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Effect of Chlorine Dioxide, Chlorite, and Nitrite on Mice with Low and High Levels of Glucose-6-Phosphate Dehydrogenase (G6PD) in their Erythrocytes

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16. ABSTRACT

Mice exposed to chlorine dioxide for 30 days at 100 ppm exhibited no significant differences from controls in any of the blood parameters measured. There were no additive or synergistic effects between chlorine dioxide and nitrite based on these same measurements.

When A/J (high G6PD) and C57L/J (low G6PD) mice were exposed to various levels of sodium chlorite (0.0, 1.0, 10.0, and 100 ppm) for 30 days in their drinking water, there were a number of blood parameters that varied significantly with respect to treatment. However, with the exception of an increase in hemoglobin for the C57L/J strain at the 1.0 and 10.0 ppm level, all significant changes were associated with the 100 ppm exposure. There were no significant strain versus treatment interactions for any level of chlorite exposure.

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise, and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The Health Effects Research Laboratory contributes to this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

This report presents data on chlorine dioxide (ClO_2), a possible alternative disinfectant for drinking water. Because of the controversy on the occurrence of carcinogenic substances (i.e. trihalomethanes) following the chlorination process, other methods of disinfection may be needed. Since chlorine dioxide may be in widespread utilization as the principal disinfectant in the United States, the intention of this study is to evaluate the health effects of chlorine dioxide and a by-product of ClO_2, sodium chlorate. In this type of research a better understanding of drinking water disinfectants is gained. Through a better understanding, potential health threats of the present and future can be identified and eliminated.

R.J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

Since chlorination of water supplies has come under investigation as a source of trihalomethane formation and possible cancer production, alternative disinfecting substances are being examined. Chlorine dioxide (ClO_2) is a leading possibility for an alternative disinfectant. However, the health consequences of ClO_2 or chlorite (a product formed in the disinfection process) are little known. Further, there is little or no information available on possible interactive effects with other oxidant compounds in the diet or water such as nitrites. The effect of these compounds might be exaggerated on red blood cells deficient in glucose-6-phosphate dehydrogenase (G6PD) and this study reports on these findings.

When mice were exposed to chlorine dioxide for 30 days at 100 ppm, there were no significant differences from controls in any of the blood parameters measured. However, significant differences did occur to MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) when receiving 50 ppm sodium nitrite in their water for 30 days. There were no strain differences nor were there any additive or synergistic effects between ClO_2 and nitrite.

Both strains of mice exposed to sodium chlorite (100 ppm), or a combination of chlorite and nitrite for 30 days experienced a number of effects on blood parameters. There were no strain versus mouse interactions indicating that the strains did not differ significantly with respect to treatment.

When A/J (high G6PD) and C57L/J (low G6PD) mice were exposed to various levels of sodium chlorite (0.0, 1.0, 10.0, and 100 ppm) for 30 days in their drinking water, there were a number of blood parameters that varied significantly with respect to treatment. However, with the exception of an increase in hemoglobin for the C57L/J strain at the 1.0 and 10.0 level, all significant changes were associated with the 100.0 ppm exposure. There were no significant strain versus treatment interactions for any level of chlorite exposure including 100 ppm.

The results indicate that chlorite produces a number of effects on erythrocytes of both A/J and C57L/J mice. Since chlorite is produced at a level of 50 percent of the chlorine dioxide demand, caution is suggested in setting a standard to insure a sufficient margin of safety. Although there is no significant difference between A/J and C57L/J mice with respect to treatment, the C57L/J (low G6PD) mice may have sufficient G6PD to overcome the oxidant stress of moderate levels of chlorite and/or nitrite. Human G6PD deficients have even less G6PD activity than the C57L/J mice and may be more sensitive than the C57L/J mouse to equivalent amounts of chlorite.

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SECTION 1

INTRODUCTION

GENERAL

The recognition that many public drinking water supplies in the United States contain carcinogenic substances has generated substantial controversy within both the scientific and lay press. The controversy has focused on the occurrence of carcinogenic substances (i.e. trihalomethanes) which tend to be formed in drinking water following the process of chlorination (1, 2, 3). Epidemiological studies have demonstrated statistical associations between increased cancer mortality and the practice of chlorination of drinking water (4, 5). These findings have tended to support laboratory studies which demonstrated the occurrence of liver cancer in selected rat and mouse strains exposed to chloroform (6). For these reasons, the EPA is considering alternative disinfectants to the process of chlorination which would decrease the trihalomethane level in drinking water.

In recognition of the potential widespread utilization of chlorine dioxide as the principal disinfectant in the United States, the intention of this study is to evaluate the health effects of chlorine dioxide and a by-product of ClO_2 disinfection, sodium chlorite, on mice with high and relatively low levels of G6PD activity in their erythrocytes.

It was predicted that such an animal model would tend to simulate the response of a possible human high risk group to these stressor agents.

Production of Chlorine Dioxide in Aqueous Solution

There are several methods by which chlorine dioxide may be efficiently produced prior to its application to the drinking water. The two principal ways by which chlorine dioxide is produced includes the following (7, 8).

a. From chlorine and sodium chlorite

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCL$$

$$HOCl + HCl + 2NaClO_2 \rightleftharpoons 2ClO_2 + NaCl + H_2O$$

b. From sodium hypochlorite and sodium chlorite

$$NaOCl + HCl \rightleftharpoons NaCl + HOCl$$

$$HCl + HOCl + 2NaClO_2 \rightleftharpoons 2ClO_2 + 2NaCl + H_2O$$

From a health point of view it is necessary to control the reaction stoichiometry and thereby prevent the release of unintended and unwanted products in the drinking water, e.g. free chlorine, chlorite, and chlorate.

Two recent EPA reports (9, 10) noted that following chlorine dioxide disinfection of surface waters, chlorites made up as much as 50 percent of the chlorine dioxide demand in the pH range of 4.8 to 9.75 while chlorates contributed from 10 to 30 percent of the chlorine dioxide demand within the same pH range. When 1.5 mg chlorine dioxide per liter (mg/l) was added to coagulated, settled, and filtered Ohio river water of 7.1 pH, a chlorite concentration of 0.72 mg/l and chlorate concentration of 0.41 mg/l resulted after 42 hours of contact. Therefore, one should expect to find varying quantities of chlorate and chlorite in water which is disinfected with chlorine dioxide. Thus, one must consider the health effects of chlorites and chlorates as well as chlorine dioxide.

Nitrite

Studies on nitrite have demonstrated its ability to produce MetHb, especially in neonates (11,12). Research has also indicated that small quantities of nitrite can generate MetHb auto-catalytically without the presence of pre-existing MetHb (13). This MetHb generated by nitrite can then serve as a catalyst for the formation of more MetHb by chlorite. Chlorite is known to oxidize hemoglobin more readily in the presence of MetHb (14).

Glucose-6-phosphate Dehydrogenase (G6PD)

In attempting to establish minimal health effect levels of oxidants on erythrocytes, it is important to consider the health effects of such stressors on high risk groups. The two largest groups that fall into this category are persons with lowered G6PD activity and neonates. G6PD deficient cells have a reduced ability to produce NADPH via the pentose phosphate pathway (PPP) and consequently less GSH is formed. Since GSH is the primary mechanism of defense of the red blood cell against oxidant stress, then persons with deficient G6PD levels have a lowered capacity for protection against oxidants (14,15,16). Neonates have a variety of deficiencies and differences in their red blood cells as compared to adults

that enhance their susceptibility to oxidant stress and methemoglobin formation (17-19). Since nitrates are used in agriculture, and occur naturally in certain food items (20-22), there is substantial opportunity for nitrates/nitrites to be ingested and so present additional oxidant stress to erythrocytes. The use of ClO_2 as a disinfectant may exaggerate these effects.

Based upon the hypothesis of the potential health effects that may be caused by chlorite and chlorite plus nitrite combinations on these high risk groups, it is logical to investigate the potential health effects of an oxidant such as ClO_2. The potential use of ClO_2 in public drinking water requires evaluation of ClO_2 alone and in combination with nitrite, with the ultimate significance being the effects of these agents on humans. Due to the overwhelming financial and ethical implications of utilizing human subjects in this study, the most logical possibility lies in the use of an animal model possessing similar enzymatic characteristics as found in the human condition so that a reasonable assessment might be made concerning the effect of ClO_2 on low level G6PD individuals.

SECTION 2

CONCLUSIONS

1. Both A/J (high G6PD activity) and C57L/J (low G6PD activity) mice exposed to 100 ppm of chlorine dioxide in their water for 30 days showed no apparent differences in all hematological parameters tested when compared with controls.

2. When exposed to 50 ppm of sodium nitrite, there was a significant decrease in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for both strains, but no additive or synergistic action was evident when 50 ppm sodium nitrite, and 100 ppm sodium chlorite were administered simultaneously.

3. A number of blood parameters were affected when mice were exposed to 100 ppm sodium chlorite and a combination of 100 ppm chlorite and nitrite for 30 days in their water. Although no strain versus treatment interactions were statistically significant, one or the other strains showed marked differences in some parameters while the other strain did not. The C57L/J strain did not appear more sensitive than the A/J strain when exposed to chlorite and chlorite plus nitrite.

4. When mice were exposed to various levels of sodium chlorite (0.0, 1.0, 10.0, 100.0 ppm) for 30 days in their drinking water, nearly all changes in parameters with respect to controls occurred in the 100 ppm exposure. At this level, both biochemical and physical measurements showed marked alterations in erythrocytes. Clearly demonstrated were the effects on red cell size where the M.C.V. and cell diameter were increased along with increased acanthocytosis and osmotic fragility.

Summary of Chlorite Effects on Erythrocytes of A/J and C57L/J Mice

Based upon the data in this report, it appears that exposure of A/J and C57L/J mice to 100 ppm of chlorite for 30 days produce increases in G6PD activity, mean corpuscular volume, osmotic fragility, acanthocytosis, and cell size (as measured by length (μ) with transmission E.M.). What specifically does this mean in terms of pathology to the erythrocyte? The

primary effect of chlorite on erythrocytes appears to be disruption of the cell membrane. There is a slight but significant increase in G6PD activity for both strains with no decrease in GSH (Table 35). This suggests that exposure to chlorite stresses the cells sufficiently to cause an increase in G6PD activity, but they were able to maintain the levels of GSH. Although GSH is important to protecting the cell against oxidative damage (23-25), the presence of GSH within the cell doesn't preclude oxidative damage to the surface membranes. Membrane damage is suggested by the evidence of increased numbers of acanthocytes in the treated mice. Acanthocytes suggest abnormal lipid content of cells (26,27) and such cells tend to become more permeable with age than normal cells (28). Peroxidative stress has been shown to increase hemolysis of such cells while substances that prevent lipid peroxidation such as tocopherol, will also prevent hemolysis (27). It seems likely, therefore, that certain oxidative drugs, including chlorite, may initiate acanthocytosis. It also seems likely that deficiencies of vitamin E might exaggerate this effect. Membrane damage as an effect of chlorite exposure is further supported by evidence of increased osmotic fragility. Increased osmotic fragility is normally associated with spherocytosis, (30,31) and the erythrocytes of persons with hereditary spherocytosis (HS) exhibit a characteristic reduction in lipid content with age when compared with normal cells (32). The HS cell also exhibits an increased rate of Na^+ flux (33) suggesting some leakiness of the membrane. The increased number of acanthocytes along with increased osmotic fragility in this study, therefore, suggest that chlorite has directly or indirectly produced damage to the erythrocyte membrane and probably has caused alteration and/or reduction of certain lipid components of the membrane. The evidence of increased mean corpuscular volume (MCV) and an increased cell length as evidenced by transmission E.M. suggest an influx of fluids to the erythrocyte. It is reasonable to suppose that membrane damage and/or reduction of ATPase activity would encourage an osmotic imbalance and increased fragility of the cell.

Although such changes in erythrocytes would likely reduce the length of survival time for the affected red cells, there was no evidence of a decreased RBC count or an increased number of reticulocytes. Therefore, the effects are not sufficient to produce a hemolytic anemia in the animals tested. Even though it was anticipated the C57L/J strain (low G6PD) would be more susceptible to chlorite ingestion, there were no obvious strain differences. There are possibly two explanations for this.

1. The primary action of chlorite is thought to be directed against the membrane of the red blood cell, and the level of G6PD activity may be unimportant in protecting these sites.

2. A prime objective of this research project was to evaluate the hypothesis that red blood cells with low levels of the enzyme G6PD are predisposed to experience biochemical changes ultimately leading to hemolysis significantly before red cells with considerably higher G6PD activity.

Human G6PD deficient individuals exhibit an enzyme activity (.6-.8 IU)* approximately 10 to 20% that of non G6PD deficient humans (2.4-3.8)*. The mouse model was selected for study because two strains were found which differed markedly in their G6PD activity with the lower strain exhibiting only 25-33% the activity of the high strain, thereby making a similar comparison of related enzyme activities between the mice strains and the deficient and normal humans. However, although the relative activities of G6PD between the low and high mice strains and humans were similar, the absolute comparisons are quite dissimilar with the low G6PD mice actually having an enzyme activity approximately the same as the normal human. Thus, if the study hypothesis is correct, then one would expect a) there should be a large number of false negatives with this model and b) considerably higher concentrations may be needed to differentiate susceptibility to chlorine dioxide and chlorite between these two strains. The lack of enhanced sensitivity of the low G6PD mouse strain suggested that higher concentrations of stressor agent would have been needed to evaluate the study hypothesis. However, since even 100 ppm chlorite was not able to cause enhanced susceptibility and that level itself exceeds by a factor of 10, what would be realistically expected to be experienced in the human situation, it did not seem reasonable to expose the animal to even higher levels of chlorite.

Thus, it would appear that the mouse model does not reveal the enhanced differential sensitivity at realistic and 10x higher than realistic concentrations to offer an effective means to evaluate the study hypothesis.

The final conclusion is that chlorite exposure in the 100 ppm range produces definite abnormalities of erythrocytes that suggest membrane damage. Such damage may occur in humans with normal G6PD levels when similarly exposed. It is possible that persons with vitamin E deficiency and/or G6PD deficiency may be at increased risk to the effects of chlorite, but this has not been demonstrated in this study. Because chlorite may be produced at a rate of 50 percent of the chlorine dioxide demand (9, 10), levels of 10 ppm have been reported under actual disinfection conditions (34). Although measurable effects may not be seen at this level, effects may be occurring that are not being measured; or high risk groups may not be part of the population being measured. In any case, the difference of 10 ppm in treatment conditions to 100 ppm where effects on erythrocytes are seen represents a safety factor of 10 or less. Normal precaution would suggest a much greater margin of safety when the number of people to be exposed represent a large portion of the population.

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*Measured as I.U. per gram of hemoglobin in whole blood hemolysate.

SECTION 3

RECOMMENDATIONS

The potential use of chlorine dioxide as an alternative disinfectant to chlorine in the nation's water supplies requires that the potential effects of its ingestion in the water supply be known. This study has shown that chlorine dioxide at 100 ppm has no measurable effect on erythrocytes of high or low G6PD mice exposed for 30 days. However, chlorite is formed at a rate of approximately 50 percent of the chlorine dioxide demand, and chlorite may reach levels of 10 ppm in communities where chlorine dioxide has had limited use.* This study reports significant effects of chlorite on the erythrocytes of A/J and C57L/J mice at 100 ppm of chlorite. This represents a factor of only 10x from the 100 ppm achieved in the supply mentioned above. Further, although the C57L/J (low G6PD) strain was predicted to be more sensitive than the A/J strain, its G6PD activity was still greater than (about 3x) a G6PD human deficient and is therefore probably more resistant to oxidant stress than the human counterpart with low G6PD activity. Therefore, the human G6PD deficient, newborns, and other human high risk groups may experience effects on erythrocytes at levels of chlorite lower than 100 ppm chlorite. Further, there may be subtle effects that occur to erythrocytes at lower levels of exposure that were not measured or considered in this study. The conclusion of these authors is that unless a safety factor greater than 10x can be achieved and demonstrated for the chlorite generated from chlorine dioxide disinfection, then use of chlorine dioxide as disinfectant may pose more serious health problems than chlorine disinfection.

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*Personal communication. Paul Heffernan, E.P.A. Cincinnati.

SECTION 4

GENERAL METHODS

ANIMAL CARE

Two strains of mice (with normal (A/J) and low levels (C57L/J) of G6PD activity) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were individually housed in stainless steel cages equipped with constant feed food hoppers and 50 ml. calibrated watering devices. The cages were kept under controlled conditions of 12-hour cycles of light and dark (9 a.m.-9 p.m. light, 9 p.m.-9 a.m. dark), and a temperature of 74±2F°. Animals were held at these conditions for a one week period after receipt in order to assess their health and allow for acclimation.

Animals were randomly allocated to each of the treatment categories, according to a random scheduling of treatments.

Treatment periods of 12 hours occurred during the dark cycle in order to minimize decomposition of ClO_2 by light and to coincide with the nocturnal activity period of the mice.

The watering devices were filled with the appropriate treatment and a reading taken of the volume both before and after the treatment period. The bottles were removed at the end of this period, and no other water provided until the next treatment period. Average daily consumption of liquid was computed for each mouse, and an overall average computed for each treatment category.

Mice were fed ad libitum a balanced rodent diet (Purina Rodent Chow) composed to not less than 23 percent crude protein, 4.5 percent crude fat, 6.0 percent crude fiber, 8.0 percent ash, and 2.5 percent minerals.

EXPERIMENTAL PROCEDURES

Blood collection and analysis

Study animals were lightly etherized and then decapitated by means of a guillotine. Blood was collected into 100 microliters of 0.6% sodium heparin (Sigma). Blood samples were then placed on ice, and aliquots were taken from these for the analysis of red blood cell parameters.

Complete blood count (C.B.C.)

The Royco Cell-crit IV Hemotology System consisting of Model 920-A Cell Counter, Model 720-A Hemoglobinometer and Model 365-A Diluter, were utilized according to specified instruction, to determine the following: red blood cell count (RBC), hematocrit (HCT), white blood count (WBC), and hemoglobin concentration (HGB). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were then computed from this data. All reagents and procedures for these tests are those approved by Royco Inc. for use with this equipment.

Quality assurance of equipment performance was checked on a daily basis by means of commercial standards, and equipment performance was measured by comparison of automated and manual hematocrits of the same blood sample.

Reticulocyte Count

The reticulocyte is a stage of development in the life of a red blood cell immediately preceding maturation of the blood cell. The number of reticulocytes is therefore an indication of the activity of erthropoietic activity. Staining of reticulocytes was accomplished according to the procedure outlined by Seiverd, 1972 (35). A 1% physiological saline solution of brilliant cresyl blue was mixed with an equal quantity of whole blood and allowed to stand for 20-30 minutes. The contents of the tube was then remixed and a smear made. The smear was observed under oil immersion and a total of 500 cells counted. The reticulocytes were expressed as a percentage of the total number of cells counted.

Glucose-6-phosphate Dehydrogenase (G6PD)

G6PD activity was determined by a colorimetric procedure using "Calbiochem" G6PD reagents and a Perkin-Elmer Coleman-55 Spectrophotometer* equipped with: 1) a flow-through temperature controlled cell; 2) a Coleman

* All colorimetric procedures in this study were read on this instrument.

5-100 Enzyme Calculator; and 3) a Coleman 47 B.C.D. Printer. The G6PD activity was calculated in terms of International Units (I.U.) where I.U.'s are defined as the activity of an enzyme which converts one micromole of substrate in one minute, under standard conditions.

The absorbance was read at 340 nanometers and 30°C for a period of 10 minutes. The change in absorbance for minutes 6 through 10 was used to calculate enzyme activity as follows:

G6PD conc. = A/5 min. x 1.00 x F x D x 1/HGB conc.

F= Temperature factor (at 30° C = 1.00)

D= Dilution factor = 4X

Osmotic Fragility (automated)

Osmotic fragility was also determined using the American Optical Fragilograph. 10 µl of whole blood was diluted in 7.5 ml of 0.68 percent isotonic buffered saline (Isoton II diluted to obtain 0.68 percent), yielding a 1:750 dilution. The instrument was calibrated with 1.5 ml of the prepared cell suspension lysed in 6 ml of deionized water. 1.5 ml of the prepared cell suspension was added to a cuvette and the osmotic fragility determined.

Deionized water was automatically added to the cell suspension in the cuvette at a rate of 0.26 ml/minute, decreasing the osmolality of the cell suspension, progressively, and producing hemolysis. Two curves were recorded: one which related light scattering by the cells during hemolysis to osmolality of the suspension medium and the second, a derivative of the first, which recorded the rate of change of light scattering as a function of the change in osmolality. An analysis of both curves permitted the determination of the points corresponding to 25, 50, and 75 percent hemolysis.

Methemoglobin assay

Methemoglobin was determined by a modification of the method of Evelyn and Malloy (36). A 120 µl aliquot of whole blood was lysed in 6 ml of M/60 phosphate buffer, centrifuges, and the hemolysate divided into four aliquots of 1.5 ml. The absorbance was read at 630 nm on a Perkin-Elmer Coleman-55 Spectrophotometer. The first aliquot was read, as is (A_1). The absorbance of aliquot two (A_2) was read two minutes after the addition of 10 µl of neutral cyanide (equal parts of 12 percent acetic acid and 10 percent aqueous sodium cyanide). The absorbance of aliquot three (A_3) was read two minutes after the addition of 10 µl of 20 percent aqueous potassium ferricyanide. Aliquot four (A_4) was incubated for two minutes after the addition of 10 µl

of 20 percent aqueous potassium ferricyanide and the absorbance read one minute after the addition of 10 µl of neutral cyanide. The calculation of methemoglobin concentration was done as follows:

$$\frac{A_1 - A_2}{A_3 - A_4} \times 100 = \text{percent methemoglobin}$$

Glutathione (GSH) assay

Reduced glutathione was determined using Glutathione An Erythrozyme Assay from Princeton Biomedix, Inc., Princeton, NJ. A 100 µl aliquot of whole blood was lysed with 2.4 ml deionized water to release the GSH content. A reagent solution containing 5,5' dithiobis - (2-nitrobenzoic acid), DTNB, which reacts with glutathione to form a highly colored yellow ion and tungstic acid to precipitate the protein was then added. The mixture was centrifuged to remove the precipitated protein and the absorbance of the supernatant determined against a deionized water blank at 412 nm on a Perkin-Elmer Coleman-55 Spectrophotometer (OD_1). OD_2 was determined exactly five minutes after the addition of 2.5 ml of the supernatant to a pre-measured vial of pH 7.0 dry phosphate buffer. The GSH in the acid supernatant reacts with the DTNB when neutralized as follows:

2 GSH + DTNB (colorless) $\rightarrow$ GSSG + 2 TNB (yellow)

The results were calculated using the following equation:

$$\text{mg GSH/ ml RBC} = \frac{OD}{11{,}000} \times \frac{5}{0.1} \times \frac{100}{HCT} \times 307.3 \times 100$$

where:

11,000 = extinction coefficient of DTNB

5 = final volume of solution in ml

0.1 = sample volume in ml

100/HCT = factor to convert blood sample volume to red cell volume

307.3 = molecular weight of glutathione

100 = factor to convert results from ml to dl

Electron Microscopy. Glutaraldehyde-Osmium fixation

All samples were fixed with freshly-prepared 2% (v/v) glutaraldehyde in 0.1 M Millonig buffer pH 7.2: (43.55) for 1h at 4C. The "fixed" pellet was washed at least twice with cold Millonig buffer and post-fixed 2h with 1% (w/v) osmium tetroxide in Millonig buffer (pH 7.2) at 4C.

Scanning Electron Microscopy

For SEM, 1 cm^2 samples were impinged onto cloth meshed fiber. The attached red blood cells were fixed in ice cold 2% (w/v) Millonig buffer - glutaraldehyde pH 7.2 for 2h; dehydrated and critical-point dried. The cells were dehydrated through a graded acetone series, and critical point-dried in a Polaron 3000 Critical-Point dryer.

The critical point-dried cells on agar surfaces were sputter-coated with a gold target to a thickness of approximately 30 nm in a Polaron E5000 Sputter Coater. The specimen stage during sputter coating was cooled with cold water to reduce surface alterations due to heating.

Electron Microscopy

All samples were examined in a Philips EM 200 electron microscope operating at 60 kv. The microscope was fitted with a liquid nitrogen anticontamination device. The electron images were recorded on Eastman fine grain release position film #5302. For scanning electron microscopy, a JEOL JSM-35 scanning electron microscope was used and the resultant images recorded on Polaroid type 665P/N film.

GENERATION OF CHLORINE DIOXIDE (ClO_2)

ClO_2 was generated on a weekly basis according to the method outlined in Standards Methods (37). The reaction that occurs in this method is as follows:

$$10\ NaClO_2 + 5\ H_2SO_4 \longrightarrow 8\ ClO_2 + 2\ HCl + 4\ H_2O \qquad (38)$$

Analytical grade reagents were utilized throughout the generation and determination phases of ClO_2 preparation. Water used in the preparation of all reagents and dilutions were processed by a "Continental Water Deionization System," consisting of two mixed bed deionizers, one activated carbon unit, a 10.0 micron prefilter and a 0.2 micron post-filter (Gelman).

Water quality of this system is continuously monitored to insure that electrical resistance never drops below 15 megohms of electrical resistance.

Determination of ClO_2 levels in solution was made by means of the Palin D.P.D. Method (39). This method utilizes the reagent diethyl-p-phenylene diamine sulphate (D.P.D.). ClO_2 and/or free chlorine will react with this reagent to form a red color. This red color can then be titrated to a colorless end point with standard Ferric Ammonium Sulfate (F.A.S) solution. For a 100 ml. sample, 1 ml. F.A.S. solution equals 1 mg./liter available chlorine or 1.9 mg./liter ClO_2.

The Palin Procedure is as follows:

Sample: 1 ml. ClO_2 stock solution to 99 ml. deionized H_2O.

To 100 ml. of sample, 2 ml. of 10% w/v Glycine solution are added, along with 0.5 grams of #1 D.P.D. Powder. This combination is then mixed at a constant speed and titrated immediately to a colorless end point. Three titrations were done daily on the stock ClO_2 solution in order to ascertain its strength. Treatment dilutions of this stock were then made up in .75 liter brown glass bottles. Those treatments containing sodium nitrite ($NaNO_2$) had the proper amount of oven dried crystals added to achieve the 50 ppm. concentration. Following preparation, the dilutions were tightly sealed and stored under refrigeration. Dilutions were then dispensed and placed on the animal cages at the beginning of the dark cycle.

STATISTICAL ANALYSIS

Analysis of variance

The analysis of variance methods rest on a separation of the variance of all the observations of an experimental design into its parts. Each of these parts measures variability attributable to some specific source, such as internal variation of the experimental populations or variation from one population to another (40,41). The expression "analysis of variance" refers to this breakdown of the sample variance. The basic question, however, concerns the comparison of the means of several populations and the parts of the sample variance are analyzed for this purpose.

In order for analysis of variance methods to be utilized effectively, two assumptions must be made: 1) that the observations in the columns and rows are random samples from a normal population and 2) that the variance σ^2, is the same for each of the normal populations (homogeneity of variance) (41). For this reason the hypothesis of equal variances, Ho: $\sigma_1^2 = \sigma_1^2 = \ldots$

$= \sigma^2_K$, is usually tested before any hypothesis testing of means. Two frequently used tests for homogeneity of variance are Bartletts and Cochrans Tests (40). A problem with these two tests is that they have lower power, i.e. the ability to reject a hypothesis that is actually false, than is desirable for most applications (41). For the practical reason of their availability on an SPSS program these tests were employed given their weaknesses.

Bartletts test requires computation with natural logarithms and provides an overall comparison of variances. Cochrans test uses the ratio of the largest variance over the sum of all the variants to evaluate the case when one variance is very much larger than the remainder of the variances. This type of deviation from homogeneous variances is serious in terms of invalidating the analysis of variance test. Before any analysis of variance was performed on the data, it was tested for homogeneity of variance by both Cochrans and Bartletts's test.

The Experimental Model

In this experiment individuals are categorized on the basis of two characteristics (animal strain and treatment) referred to as S and T categories. Since the S and T categories exhaust all the categories of the two variables, the experimental models considered a fixed-constants of model I situation. The fixed effects two-way layout is: $yijk = \mu + \alpha i + \beta j + (\alpha\beta) ij + eijk$ where μ is the overall mean, αi is the i^{th} main effect of factor S and βj is the j^{th} main effect of factor T. The quantity $(\alpha\beta)ij$ is called the two way interaction of the i^{th} level of S and the j^{th} level of T and $eijk$ is the independent error variable. The two way ANOVA table for this model show sources of variation from factors S, T, the interaction of S and T, and the residual (42). The three hypotheses usually tested are:

Ho: all $\alpha i = o$ (no factor R main effects)

Ho: all $\beta j = o$ (no factor C main effects)

Ho: all $(\alpha\beta)ij = o$ (no interaction effects)

If any of these hypotheses are rejected it can be concluded that at least one cell mean is significantly different but one cannot be more specific. Contrasts of individual cell means can be constructed with coefficients in the linear expression $a_1 \mu_2 + a_2 \mu_2 + \ldots + a_k \mu_k$ where $a_1 + a_2 + \ldots + a_k = o$ (44). Multiple contrasts can be constructed and are called orthogonal if in addition to $\Sigma ai = o$, $\Sigma bi = o$, etc. that $\Sigma aibi = o$. If this additional factor is satisfied then overall power can be maintained. Where analysis of variance showed significance for treatment (T), differences strain (5) differences, or strain vs. treatment (ST) interaction: orthagonal contrasts were used to determine which specific treatment or strain

contributed most to the significant differences.

Computer Programs

Analysis was performed using the BMDP2V (43) and SPSS ONEWAY (44) programs. The two way ANOVA table was produced by the BMDP program with the three previously mentioned hypotheses either accepted or rejected based upon the p-values of the F test ($\alpha = .05$). If a hypothesis was rejected, contrasts were constructed and with separate variances computed with accompanying p-values. These contrasts were run on the SPSS ONEWAY program (44).

The Bartletts and Cochrans tests for equal variances were also computed using the SPSS ONEWAY program.

SECTION 5

EXPOSURE OF A/J and C57L/J MICE TO CHLORINE DIOXIDE, (ClO_2), SODIUM NITRITE, (NO_2-), AND A COMBINATION OF BOTH FOR 30 DAYS

A/J and C57L/J mice were divided into four groups with groups receiving 100 ppm ClO_2; or 50 ppm of sodium nitrite; or 100 ppm ClO_2 and 50 ppm nitrite; or control mice receiving deionized water. The groups were treated for 30 days at which time the mice were sacrificed and analyses performed on heparinized blood. The results of these treatments are listed and described in the following Tables 1-11 and Figures 1-2 for each hematologic parameter tested. A discussion of the results follows.

A number of differences in hematologic parameters between the C57L/J and A/J strains was indicated by Hutton in 1972 (45). These reported differences include a greater RBC and HCT for the C57L/J strain, while the A/J strain has a G6PD activity approximately three-fold that of the C57L/J strain. These findings were consistently observed throughout this study. In the present study, the C57L/J showed greater levels of hemoglobin; a larger mean corpuscular volume; and more osmotically fragile cells than the A/J strain. The mean corpuscular hemoglobin concentration (MCHC) was greater for the A/J strain than the C57L/J strain. These differences were statistically significant at $p = .05$ or greater.

A total of 11 hematologic parameters were measured for effects of chlorite and/or nitrite. There were no strain versus treatment interactions for any parameters indicating that the C57L/J strain did not respond differently than the A/J strain to any of the treatments.

Two hematologic parameters, MCH (Table 7, Fig. 1) and MCHC (Table 8, Fig. 2) varied significantly with treatment ($p = .032$, and 0.40 respectively). When the cell means are examined, the lowest values for MCH and MCHC for both strains occur with treatment 2 (0.0 ppm ClO_2, 50 ppm NCl_2-). The percentage change from controls using cell means was determined for treatment 2, 3, and 4 for MCH (Fig. 1) and MCHC (Fig. 2). The MCH for the A/J strain decreased over 9 percent ($p = .004$) for treatment 1 (50 ppm NO_2-) while treatments 3 and 4 did not differ markedly from the control but differed significantly from treatment 2. The C57L/J strain showed a slight decrease from controls in treatment 2 while increasing for treatment 3 (6.6%) and treatment 4 (9.1%). However, contrasts failed to show

a significant difference of treatments 2, 3, or 4 from controls (treatment 1). Similar results were obtained for MCHC (Fig. 2). The conclusion, therefore, is that the only treatment to significantly effect MCH of MCHC values is a 30 day exposure to 50 ppm of sodium nitrite. Chlorine dioxide alone or in combination with nitrite had no effect on any of the 11 hematologic parameters measured in the study.

TABLE 1

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR ENZ (GLUCOSE-6-PHOSPHATE DEHYDROGENASE) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - ENZ

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	455.79851	1	23008.62542	.000
S	38.47864	1	1942.42900	.000
T	.05209	3	.87646	.456
ST	.013	3	.2210	.682
ERROR	2.02057	102		

B. Descriptive Statistics

Variable Number	1	Maximum	8.63
Name . . . . . ENZ		Minimum	1.39
Number of Distinct Values .	104	Range	7.24
Number of Values Counted .	126	St. Dev.	2.41
Number of Values not Counted	0		

Location Estimates

Mean	4.35
Median	4.33
Mode	NOT UNIQUE

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	6.3	2.06
2	0	50	6.4	1.88
3	100	0	7.13	2.43
4	100	50	6.55	2.01

TABLE 2

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR RBC (RED BLOOD CELL COUNT) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - RBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	6705.51225	1	15796.78478	.000
S	25.35002	1	59.71935	.000
T	.57581	3	.45216	.716
ST	1.07864	3	.84702	.471
ERROR	3.29756	102		

B. Descriptive Statistics

Variable Number	2	Maximum	9.15
Name	RBC	Minimum	5.44
Number of Distinct Values .	99	Range	3.71
Number of Values Counted .	126	St. Dev.	.81
Number of Values not Counted	0		

Location Estimates

Mean	7.65
Median	7.72
Mode	7.72

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	7.48	8.15
2	0	50	8.08	8.31
3	100	0	7.48	8.09
4	100	50	7.31	8.03

TABLE 3

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR HCT (HEMATOCRIT) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - HCT

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	289.05446	1	194519.62307	.000
S	.11734	1	72.23767	.000
T	.00146	3	.32673	.806
ST	.00175	3	.39323	.758
ERROR	.15157	102		

B. Descriptive Statistics

Variable Number	3	Maximum	53.90
Name . . . . . HCT		Minimum	27.15
Number of Distinct Values .	99	Range	26.75
Number of Values Counted .	126	St. Dev.	4.37
Number of Values not Counted	0		

Location Estimates

Mean	38.39
Median	39.17
Mode	NOT UNIQUE

C. Cell Means

#	Treatment (ppm) ClO_2	Nitrite	Strain A/J	C57L/J
1	0	0	37.21	40.43
2	0	50	39.47	42.65
3	100	0	38.20	41.19
4	100	50	37.12	40.34

TABLE 4

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR WBC (WHITE BLOOD CELL COUNT) OF A/J and C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - WBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	58.18724	1	2015.49173	.000
S	.13962	1	4.83617	.030
T	.02390	3	.27593	.843
ST	.03493	3	.40334	.751
ERROR	2.94474	102		

B. Descriptive Statistics

Variable Number	4	Maximum	19.20
Name . . . . . WBC		Minimum	1.65
Number of Distinct Values .	77	Range	17.55
Number of Values Counted .	126	St. Dev.	2.46
Number of Values not Counted	0		

Location Estimates

Mean	5.50
Median	5.07
Mode	NOT UNIQUE

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	4.55	4.27
2	0	50	5.05	4.37
3	100	0	4.54	3.4
4	100	50	6.22	5.28

TABLE 5

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR HGB (HEMOGLOBIN) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - HGB

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	20616.00240	1	29488.61112	.000
S	52.93168	1	75.71215	.000
T	3.91658	3	1.86739	.140
ST	3.10161	3	1.47882	.225
ERROR	71.30998	102		

B. Descriptive Statistics

Variable Number	5	Maximum	15.75
Name . . . . . HGB		Minimum	9.80
Number of Distinct Values .	68	Range	5.95
Number of Values Counted .	126	St. Dev.	1.14
Number of Values not Counted	0		

Location Estimates

Mean	13.49
Median	13.60
Mode	13.55

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	13.40	13.61
2	0	50	13.11	13.45
3	100	0	13.47	14.28
4	100	50	13.45	14.64

TABLE 6

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MCV (MEAN CORPUSCULAR VOLUME) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCV

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	57.13653	1	135061.49338	.000
S	.00175	1	4.12639	.045
T	.00032	3	.24899	.862
ST	.00103	3	.81376	.489
ERROR	.04315	102		

B. Descriptive Statistics

Variable Number	6	Maximum	63.5
Name . . . . . MCV		Minimum	43.5
Number of Distinct Values .	54	Range	20.0
Number of Values Counted .	126	St. Dev.	2.58
Number of Values not Counted	0		

Location Estimates

Mean	50.7
Median	50.4
Mode	49.5

C. Cell Means (Log)

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	49.72	49.63
2	0	50	48.95	51.32
3	100	0	50.51	50.94
4	100	50	49.02	50.22

TABLE 7

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MCH (MEAN CORPUSCULAR HEMOGLOBIN) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCH

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	35524.48350	1	28274.28819	.000
S	3.22067	1	2.56337	.112
T	11.48141	3	3.04605	.032
ST	2.12864	3	.56337	.639
ERROR	128.15521	102		

B. Descriptive Statistics

Variable Number	7	Maximum	20.19
Name	MCH	Minimum	14.65
Number of Distinct Values .	108	Range	5.54
Number of Values Counted .	126	St. Dev.	1.15
Number of Values not Counted	0		

Location Estimates

Mean	17.66
Median	17.53
Mode	NOT UNIQUE

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	17.91	16.72
2	0	50	16.27	16.18
3	100	0	18.09	17.75
4	100	50	17.82	18.25

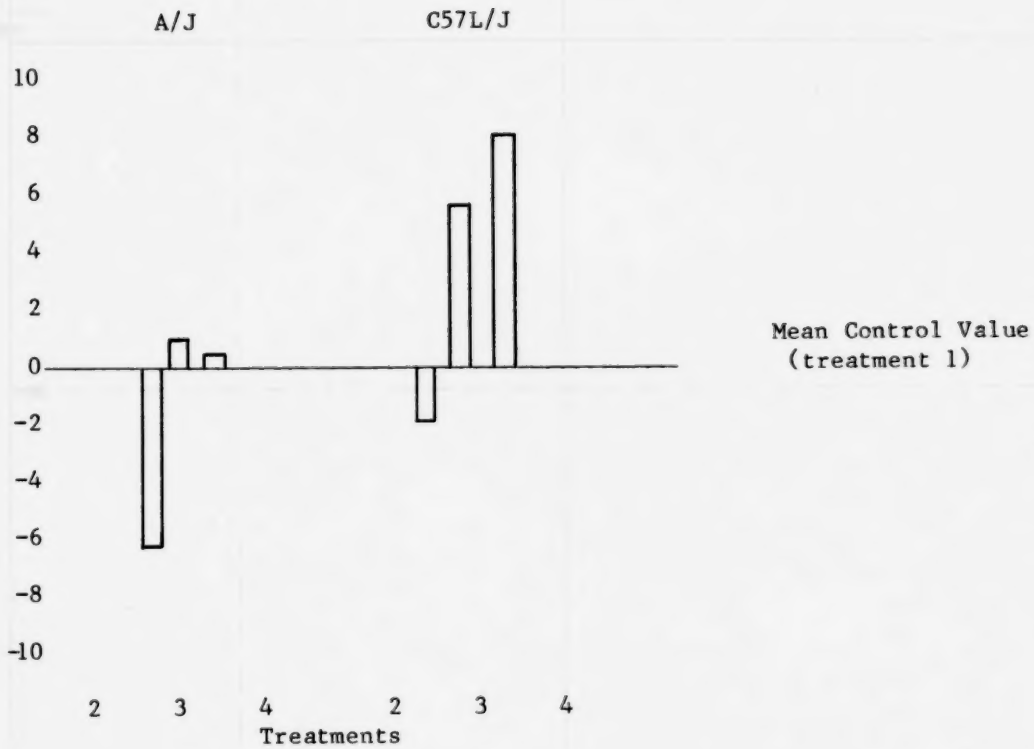

Figure 1. Percent change from controls of Mean Corpuscular Hemoglobin (MCH) for A/J and C57L/J mice exposed to ClO_2, NO_2^-, and ClO_2 plus NO_2^- for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of this Difference Occurring by Chance Alone
A/J	1 - 2	.004
	2 - 3	.002
	2 - 4	.021
C57L/J	2 - 2	.018
	2 - 4	.000

TABLE 8

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MCHC (MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCHC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	138087.36309	1	24133.60571	.000
S	47.29079	1	8.26504	.005
T	49.33010	3	2.87382	.040
ST	3.20218	3	.18655	.905
ERROR	583.62232	102		

B. Descriptive Statistics

Variable Number	8	Maximum	40.33
Name	MCHC	Minimum	26.81
Number of Distinct Values .	117	Range	13.52
Number of Values Counted .	126	St. Dev.	2.58
Number of Values not Counted	0		

Location Estimates

Mean	34.86
Median	34.78
Mode	33.93

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	36.01	33.70
2	0	50	33.25	31.53
3	100	0	35.85	34.81
4	100	50	36.30	36.36

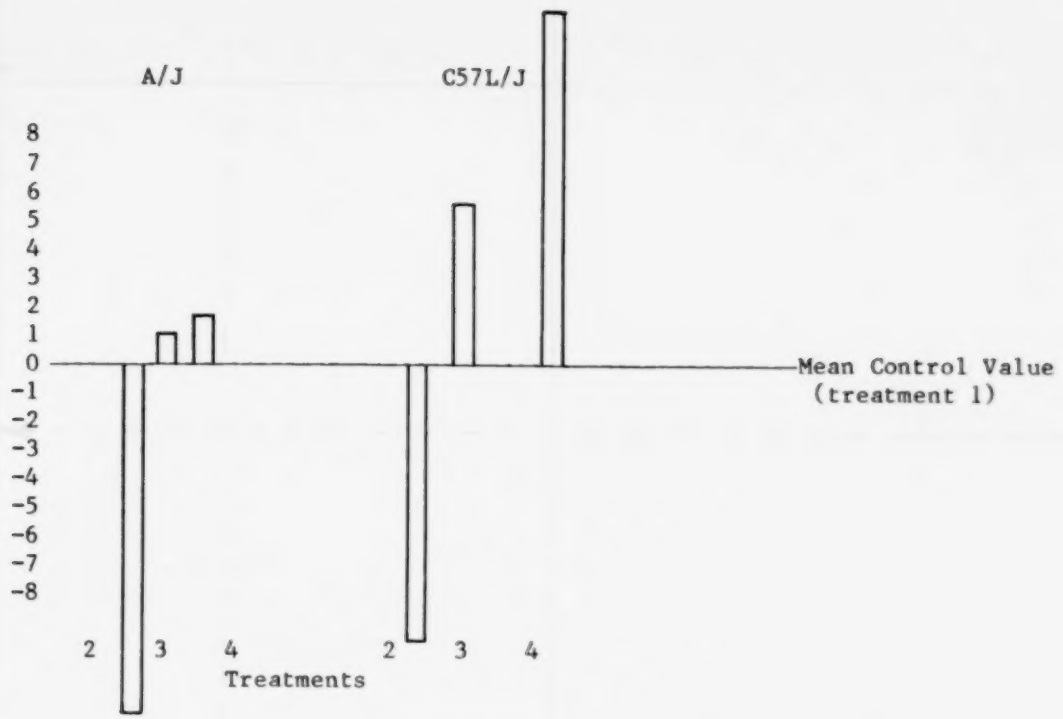

Figure 2. Percent change from controls of Mean Corpuscular Hemoglobin Concentration (MCHC) for A/J and C57L/J mice exposed to ClO_2, NO_2^-, and ClO_2 plus NO_2^- for 30 days.

CONTRASTS

Strain	Treatment Contrasted	Probability of the Difference Occurring by Chance Alone
A/J	1 - 2	.003
	2 - 3	.015
	2 - 4	.007
C57L/J	2 - 3	.011
	2 - 4	.000

TABLE 9

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR RET (RETICULOCYTES) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - RET

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	.51257	1	4.09457	.046
S	.09086	1	.72581	.396
T	.21294	3	.56699	.638
ST	.25306	3	.67384	.570
ERROR	11.89249	95		

B. Descriptive Statistics

Variable Number	9	Maximum	9.9
Name	RET	Minimum	0.0
Number of Distinct Values .	21	Range	9.9
Number of Values Counted .	120	St. Dev.	2.1
Number of Values not Counted	6		

Location Estimates

Mean	1.74
Median	1.00
Mode	NOT UNIQUE

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	2.4	1.20
2	0	50	1.15	0.53
3	100	0	1.00	1.07
4	100	50	1.58	.91

TABLE 10

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR OSM (OSMOTIC FRAGILITY) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - OSM

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	7.74535	1	29090.12069	.000
S	.01004	1	37.71023	.000
T	.00045	3	.55809	.644
ST	.00102	3	1.28271	.284
ERROR	.02716	102		

B. Descriptive Statistics

Variable Number	9	Maximum	.627
Name . . . . . OSM		Minimum	.487
Number of Distinct Values .	31	Range	.140
Number of Values Counted .	126	St. Dev.	.025
Number of Values not Counted	0		

Location Estimates

Mean	.550
Median	.550
Mode	.541

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	.541	.549
2	0	50	.522	.559
3	100	0	.507	.533
4	100	50	.538	.558

TABLE 11

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MTHB (METHEMOGLOBIN) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MTHB

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	1.51232	1	19.11101	.022
S	.01867	1	.23594	.660
T	.02573	1	.32514	.608
ST	.01867	1	.23594	.660
ERROR	.23740	3		

B. Descriptive Statistics

Variable Number	10	Maximum	.92
Name . . . . . MTHB		Minimum	0
Number of Distinct Values .	7	Range	.92
Number of Values Counted .	126	St. Dev.	.13
Number of Values not Counted	0		

Location Estimates

Mean	.294
Median	0.000
Mode	0.000

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	.450	.450
2	0	50	.470	.700
3	100	0	.265	.311
4	100	50	0.00	0.00

SECTION 6

EXPOSURE OF A/J AND C57L/J MICE TO CHLORITE, AND CHLORITE PLUS NITRITE FOR 30 DAYS

Both A/J and C57L/J (N=132) were exposed to 100 ppm of chlorite, or 100 ppm chlorite plus 50 ppm nitrite for 30 days in their drinking water. The chlorite and nitrite were weighed and dissolved in deionized water to yield the appropriate concentrations. Controls were given deionized water for the 30 days. Water consumption was measured daily for each animal.

G6PD ENZYME ACTIVITY (TABLE 12, FIGURE 3)

Analysis of variance revealed both strain (p=.000) and treatment (p=.000) differences. The C57L/J strain was again, as expected, approximately one-third the G6PD activity of the A/J strain (Table 15). The G6PD enzyme activity was increased for treatment 2 (100 ppm chlorite) and 4 (100 ppm chlorite and 50 ppm nitrite) for both strains with the major and significant difference from controls being a 22.5 percent increase for the C57L/J strain exposed to a combination of nitrite and chlorite (p=.003) (Figure 15). This was significantly greater than the mice receiving chlorite alone, while nitrites have been shown (Table 1, Figure 1) not to significantly affect G6PD levels. In this case, nitrates appeared to exaggerate the effect of chlorites on G6PD activity of C57L/J mice. In terms of an explanation, it ppears that the C57L/J mice responded to oxidant stress by increasing the G6PD activity.

RBC (TABLE 13)

The average red blood cell count of the C57L/J strain is significantly greater than that of the A/J strain (p=.000). This is a consistent finding in this report and in keeping with the reported literature (45). Analysis of variance indicates significant differences with respect to treatment. However, when contrasts are used to isolate treatments, no significant differences occur for treatments 2 or 3. The lack of treatment effect is evident when examining the cell means for control and treatment groups.

HCT (TABLE 14, FIGURE 4)

The hematocrit of the C57L/J is significantly higher (p=.000) than the A/J strain, a finding consistent with other sections in this report and the literature (45). A treatment effect is significant at the p = .003 level. An examination of the means, percentage change fron controls, and contrasts reveals that the HCT increases significantly (p=.001) from controls only for the C57L/J strain treated with 100 ppm chlorite.

WBC (TABLE 15)

Analyses of variance shows no strain, treatment, or strain vs. treatment differences as significant.

HGB (TABLE 16, FIGURE 5)

The mean hemoglobin level of the C57L/J strain is significnatly greater than the A/J strain. The A/J strain alone shows a significant reduction with chlorite (p=.002), and chlorite plus nitrite (p=.010).

MCV (TABLE 17, FIGURE 6

The average mean corpuscular volume of erythrocytes of the C57L/J strain are greater than that of A/J strain and is consistent with the literature (45). Treatment with chlorite or chlorite plus nitrite produces significant increases in MCV for both A/J and C57L/J strains. The strains of mice behave similarly with respect to treatment, and nitrites do not exaggerate the effects of chlorite on the MCV.

MCH (TABLE 18)

The A/J strain shows a significantly greater mean corpuscular hemoglobin than the C57L/J strain (p=.004). There are no treatment or strain vs. treatment differences from controls.

MCHC (TABLE 19)

The mean corpuscular hemoglobin concentration of the A/J strain is significantly greater than the C57L/J strain. Although the MCHC decreases with treatment for both strains for treatments 2 and 3, neither analysis of variance or contrasts shows significance beyond the p = .05 level.

RET (TABLE 20)

There are no significant differences for strain, treatment, or strain vs. treatment groups when compared with controls for reticulocyte counts. Further, a maximum count of nine percent with a mean of .07 percent indicates most reticulocyte counts were below one percent. These levels are not clinically important.

OSM (TABLE 21, FIGURE 7)

Osmotic fragility of the erythrocytes was greater for the C57L/J strain than the A/J strain. This finding is consistent through this study and consistent with the reported literature (45). Further, the A/J strain showed a significant increase in osmotic fragility from controls 1-5 only for the chlorite treatment ($p=.018$). The presence of nitrites reduced the effect of chlorites on the MCV of the A/J strain ($p=.038$).

MTHB (TABLE 22)

Methemoglobin levels did not vary significantly between strains ($p=.419$). There appeared to be a difference in treatment effects on MTHB levels ($p=.054$), but the maximum level of METHB attained was 11.5 percent and with a median value of 0.00 percent, it suggested that most METHB levels were not detectable. Therefore, no clinical importance may be attached to the levels of MTHB achieved in this experiment.

WAT (TABLE 23)

The amount of water consumed by the mice did not vary between strains, and treatment had no effect on the amount consumed when compared with controls. Therefore, it appears that even 100 ppm of chlorite in the drinking water did not effect the organoleptic properties to cause the mice to consume less, and water consumption was not a factor in treatment effects

A compilation of the above data reveals that treatment of A/J and C57L/J mice with 100 ppm ClO_2^- or 100 ppm ClO_2^- plus 50 ppm NO_2^- for 30 days resulted in significant treatment effects for five of the 11 variables tested (Table 24). These variables include ENZ, HCT, HGB, MCV, and OSM. Since nitrites were shown to primarily effect MCH and MCHC (Tables 7 and 8, Figures 1 and 2) all of the treatment effects listed here may be attributed to chlorite. Because chlorite exposure did produce the main effects, the next phase of the study was designed to evaluate the dose-response relationship of chlorites to effects on various hematologic parameters.

TABLE 12

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR G-6-PD ACTIVITY (ENZ) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - ENZ

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	46453.78504	1	18950.69404	.000
S	3468.82561	1	1415.09788	.000
T	33.12840	3	4.50488	.005
ST	4.15843	3	.56547	.639
ERROR	272.09400	111		

B. Descriptive Statistics

Variable Number	3	Maximum	8.30
Name	ENZ	Minimum	1.47
Number of Distinct Values .	121	Range	6.83
Number of Values Counted .	135	St. Dev.	2.29
Number of Values not Counted	0		

Location Estimates

Mean	4.25
Median	3.08
Mode	1.78

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	6.32	2.06
2	100	0	6.82	2.16
3	100	50	6.53	2.53

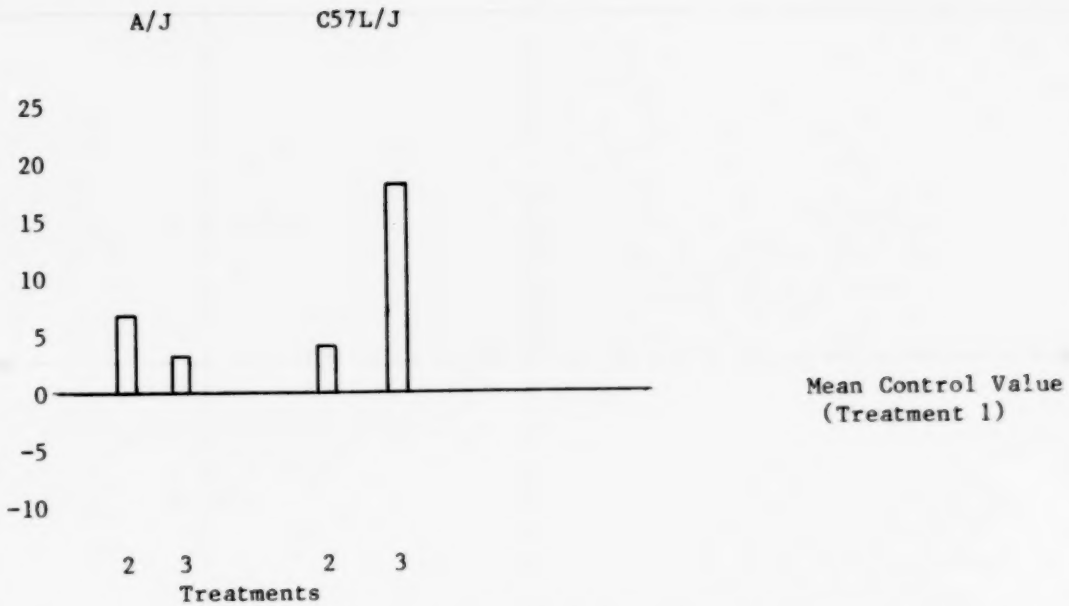

Figure 3. Percentage change from controls for G6PD activity of A/J and C57L/J mice exposed to chlorite, or chlorite plus nitrite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of Difference Occurring by Chance Alone
A/J	- - - -	- - - -
C57L/J	1 vs. 3	0.003
	2 vs. 3	0.032

TABLE 13

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR RED BLOOD CELL COUNT (RBC) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - RBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	6594.57445	1	17789.83829	.000
S	30.57933	1	82.49226	.000
T	3.79384	3	3.41148	.020
ST	.85050	3	.76479	.516
ERROR	41.14696	111		

B. Descriptive Statistics

Variable Number	2	Maximum	9.40
Name . . . . . RBC		Minimum	5.24
Number of Distinct Values .	108	Range	4.16
Number of Values Counted .	135	St. Dev.	.86
Number of Values not Counted	0		

Location Estimates

Mean	7.66
Median	7.70
Mode	NOT UNIQUE

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	7.48	8.14
2	100	0	7.24	8.50
3	100	50	7.59	8.14

TABLE 14

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR HEMATOCRIT (HCT) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - HCT

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	179463.04681	1	17440.77663	.000
S	1245.69378	1	121.06039	.000
T	150.688833	3	4.88145	.003
ST	3.50383	3	.11350	.952
ERROR	1142.17380	111		

B. Descriptive Statistics

Variable Number	3	Maximum	56.15
Name . . . . . HCT		Minimum	26.93
Number of Distinct Values .	99	Range	29.22
Number of Values Counted .	135	St. Dev.	4.96
Number of Values not Counted	0		

Location Estimates

Mean	40.00
Median	40.50
Mode	37.00

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	37.2	40.4
3	100	1	37.4	43.8
4	100	50	38.8	42.1

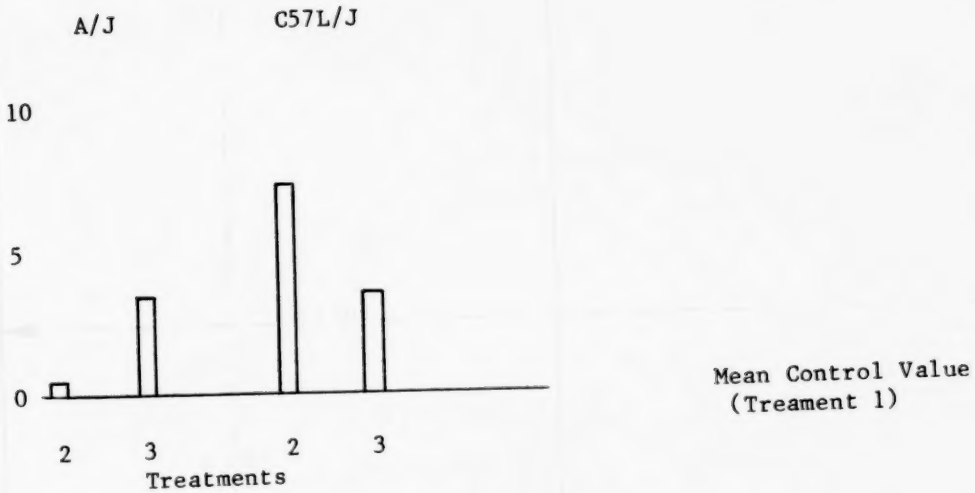

Figure 4. Percentage change from controls for Hematocrit (HCT) of A/J and C57L/J mice exposed to chlorite, or chlorite plus nitrite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of Difference Occurring by Chance Alone
C57L/J	1 vs. 2	.001

TABLE 15

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR WHITE BLOOD COUNT (WBC) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - WBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	2788.43728	1	443.65087	.000
S	13.04309	1	2.07520	.153
T	21.55071	3	1.14293	.335
ST	11.33147	3	.60096	.616
ERROR	697.65791	111		

B. Descriptive Statistics

Variable Number	4	Maximum	22.75
Name . . . . . WBC		Minimum	1.45
Number of Distinct Values .	87	Range	21.30
Number of Values Counted .	135	St. Dev.	2.54
Number of Values not Counted	0		

Location Estimates

Mean	4.81
Median	4.40
Mode	3.60

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	4.57	4.26
2	100	0	3.14	4.61
3	100	50	3.33	5.69

TABLE 16

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR HEMOGLOBIN (HGB) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - HGB

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	20328.75314	1	33381.54508	.000
S	56.29622	1	92.44318	.000
T	10.77233	3	5.89636	.001
ST	3.07959	3	1.68565	.174
ERROR	67.59698	111		

B. Descriptive Statistics

Variable Number	5	Maximum	15.80
Name . . . . . HGB		Minimum	10.45
Number of Distinct Values .	65	Range	5.35
Number of Values Counted .	135	St. Dev.	1.16
Number of Values not Counted	0		

Location Estimates

Mean	13.35
Median	13.35
Mode	14.60

C. Cell Means

#	Treatment (ppm) ClO_2	Nitrite	Strain A/J	C57L/J
1	0	0	13.4	13.6
2	100	0	12.6	14.2
3	100	50	12.7	13.8

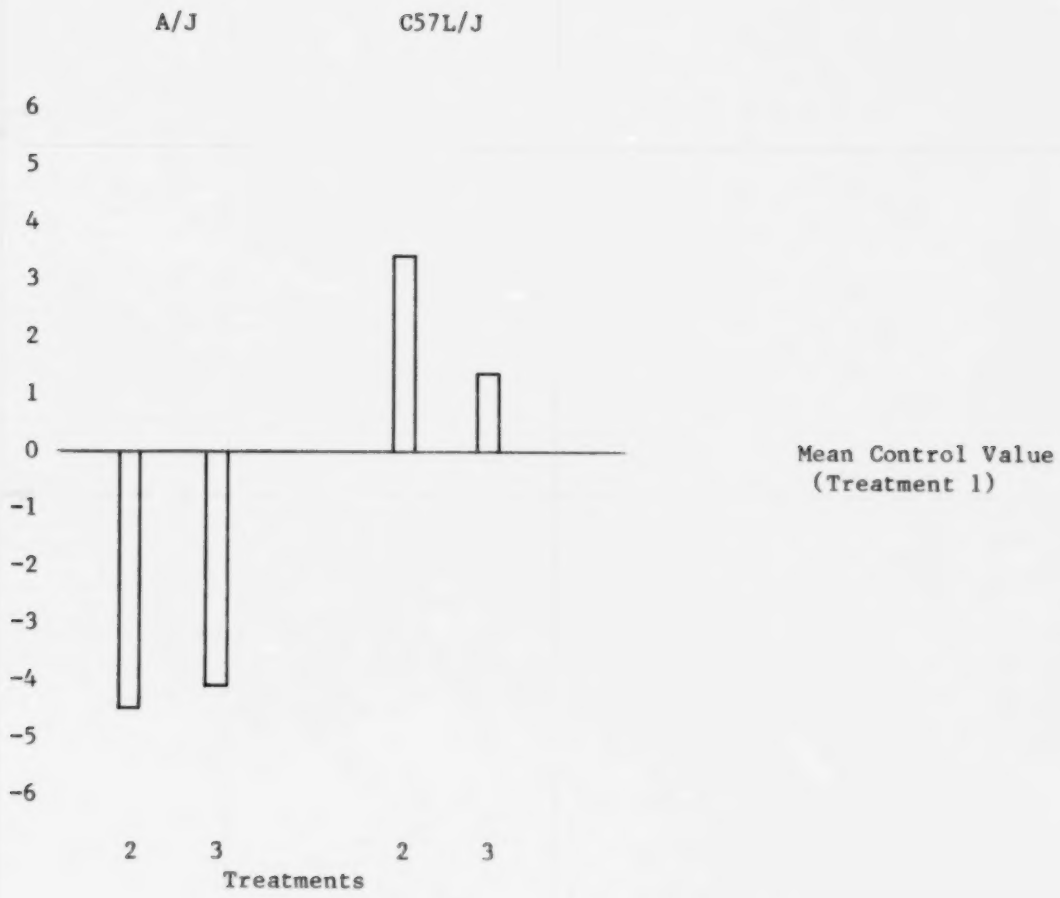

Figure 5. Percentage change from controls for HGB (Hemoglobin) of A/J and C57L/J mice exposed to chlorite, or chlorite plus nitrite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of Difference Occurring by Chance Alone
A/J	1 vs. 2	.002
	1 vs. 3	.010

TABLE 17

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MEAN CORPUSCULAR VOLUME (MCV) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCV

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	314116.23372	1	25768.95491	.000
S	60.03611	1	4.92514	.029
T	99.48134	3	2.72036	.048
ST	17.65645	3	.48282	.695
ERROR	1353.05844	111		

B. Descriptive Statistics

Variable Number	5	Maximum	63.87
Name . . . . . MCV		Minimum	46.91
Number of Distinct Values .	127	Range	16.96
Number of Values Counted .	135	St. Dev.	3.89
Number of Values not Counted	0		

Location Estimates

Mean	52.30
Median	51.07
Mode	NOT UNIQUE

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	49.74	49.62
2	100	0	51.8	51.60
3	100	50	51.07	51.8

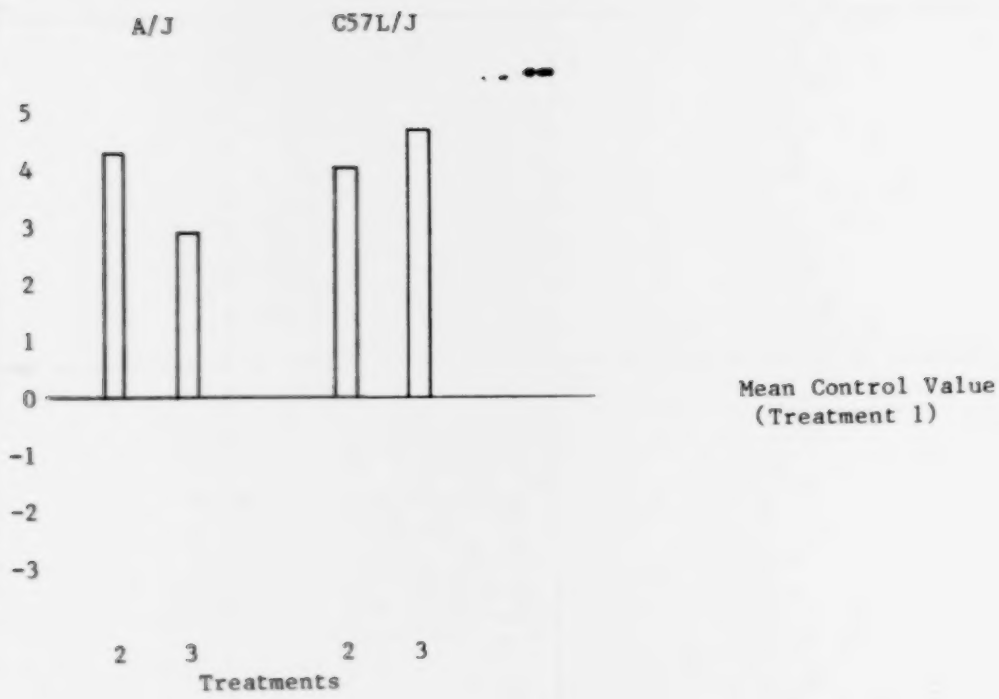

Figure 6. Percentage change from controls for MCV (Mean Corpuscular Volume) of A/J and C57L/J mice exposed to chlorite, or chlorite plus nitrite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of Difference Occurring by Chance Alone
A/J	1 vs. 2	0.029
C57L/J	1 vs. 2	0.018
	1 vs. 3	0.015

TABLE 18

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MEAN CORPUSCULAR HEMOGLOBIN (MCH) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2, NO_2^-, OR ClO_2 PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCH

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	35816.86007	1	31175.26755	.000
S	10.18028	1	8.86099	.004
T	.33501	3	.09720	.961
ST	.45661	3	.13248	.941
ERROR	127.52646	111		

B. Descriptive Statistics

Variable Number	7	Maximum	20.42
Name	MCH	Minimum	15.32
Number of Distinct Values .	112	Range	5.10
Number of Values Counted .	135	St. Dev.	1.23
Number of Values not Counted	0		

Location Estimates

Mean	17.51
Median	17.36
Mode	18.46

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	17.91	16.72
2	100	0	17.46	16.71
3	100	50	16.46	17.02

TABLE 19

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MCHC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	131957.54968	1	31092.56836	.000
S	130.58961	1	30.77025	.000
T	32.37143	3	2.54251	.060
ST	9.12589	3	.71676	.544
ERROR	471.08646	111		

B. Descriptive Statistics

Variable Number	8	Maximum	40.97
Name	MCHC	Minimum	28.14
Number of Distinct Values .	123	Range	12.83
Number of Values Counted .	135	St. Dev.	2.42
Number of Values not Counted	0		

Location Estimates

Mean	33.57
Median	33.30
Mode	NOT UNIQUE

C. Cell Means

	Treatment (ppm)		Strain	
#	ClO_2	Nitrite	A/J	C57L/J
1	0	0	36.00	33.70
2	100	0	33.70	32.39
3	100	50	32.82	32.83

TABLE 20

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR RETICULOCYTES (RET) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - RET

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	114.43543	1	43.64545	.000
S	.937641	1	.35762	.551
T	9.11568	3	1.15890	.329
ST	19.00431	3	2.41607	.070
ERROR	291.03454	111		

B. Descriptive Statistics

Variable Number		3	Maximum	9.0
Name	RET	9	Minimum	0.0
Number of Distinct Values .		16	Range	9.0
Number of Values Counted .		132	St. Dev.	1.06
Number of Values not Counted		3		

Location Estimates

Mean	.07
Median	.05
Mode	.02

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	1.15	1.20
2	100	0	0.69	0.99
3	100	50	0.74	0.55

TABLE 21

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR OSMOTIC FRAGILITY (OSM) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - OSM

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	3467.80579	1	133397.02828	0.000
S	.71837	1	27.63391	.000
T	.36410	3	4.66863	.004
ST	.06961	3	.89262	.447
ERROR	2.88557	111		

B. Descriptive Statistics

Variable Number	10	Maximum	.609
Name . . . . . OSM		Minimum	.490
Number of Distinct Values .	35	Range	.119
Number of Values Counted .	135	St. Dev.	.196
Number of Values not Counted	0		

Location Estimates

Mean	.549
Median	.550
Mode	.550

C. Cell Means

#	Treatment (ppm) ClO_2	Nitrite	Strain A/J	C57L/J
1	0	0	.522	.559
2				
3	100	1	.547	.558
4	100	50	.535	.550

Figure 7. Percentage change from controls for Osmotic Fragility (OSM) of A/J and C57L/J mice exposed to chlorite, or chlorite plus nitrite for 30 days.

CONTRASTS

Strain	Treatment Contrasted	Probability of Difference Occurring by Chance Alone
A/J	1 vs. 2	0.018
	2 vs. 3	0.038

TABLE 22

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR MTHB (METHEMOGLOBIN) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - MTHB

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	5.70770	1	38.94384	.000
S	.09652	1	.65854	.419
T	1.15304	3	2.62242	.054
ST	.21809	3	.49602	.686
ERROR	16.26842	111		

B. Descriptive Statistics

Variable Number	11	Maximum	1.50
Name . . . . . MTHB		Minimum	.00
Number of Distinct Values .	43	Range	1.50
Number of Values Counted .	135	St. Dev.	.38
Number of Values not Counted	0		

Location Estimates

Mean	.24
Median	.00
Mode	.00

C. Cell Means

#	Treatment (ppm) ClO_2	Treatment (ppm) Nitrite	Strain A/J	Strain C57L/J
1	0	0	.11	.15
2	100	0	.34	.25
3	100	50	.27	.36

TABLE 23

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR WAT (AVERAGE ML OF WATER CONSUMED PER DAY) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO ClO_2^-, NO_2^-, OR ClO_2^- PLUS NO_2^-

A. Analysis of Variance for 1 -ST Dependent Variable - WAT

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	66772.17	1	16695.79	.000
S	3.10	1	.77	.381
T	5.85	3	.488	.691
ST	3.64	3	.303	.823
ERROR	443.93	111		

B. Descriptive Statistics

Variable Number	21	Maximum	9.79
Name . . . . . WAT		Minimum	2.97
Number of Distinct Values .	90	Range	6.86
Number of Values Counted .	135	St. Dev.	.98
Number of Values not Counted	0		

Location Estimates

Mean	5.94
Median	5.86
Mode	7.07

C. Cell Means

#	Treatment (ppm) ClO_2	Nitrite	Strain A/J	C57L/J
1	0	0	6.01	5.29^2
2	100	0	6.0	6.6^4
3	100	50	6.1	6.0^4

TABLE 24

VARIABLES AND STRAINS FOR WHICH TREATMENTS 1 (100 PPM CHLORITE) AND 2 (100 PPM CHLORITE, 50 PPM NITRITE) PRODUCED SIGNIFICANT EFFECTS COMPARED WITH CONTROLS

VARIABLES	A/J STRAIN		C57L/J STRAIN	
	100 ppm*	100 ppm 50 ppm**	100 ppm*	100 ppm 50 ppm**
ENZ	-	-	-	.003†>
HCT	-	-	.001>	-
HGB	.002††<	.010>	-	-
MCV	.029>	-	.018>	.015>
OSM	.018>	-	-	-

*100 ppm ClO_2^-

** 50 ppm NO_2^-

† 'P' values determined by orthagonal contrasts

†† Direction of change from control: > = higher mean value than control; < = lower mean value than control.

SECTION 7

EXPOSURE OF A/J AND C57L/J MICE TO 0.0, 1.0, 10.0, and 100.0 PPM OF CHLORITE FOR 30 DAYS

EFFECT ON HEMATOLOGICAL PARAMETERS

Both A/J and C57L/J strains of mice were exposed to 0.0, 1.0, 10.0, and 100.0 ppm of sodium chlorite in their drinking water for 30 days. The mice were then sacrificed, the blood collected in heparin, and the various blood parameters measured. The results appear in Tables 25-37 and Figures 8-13. Consistent with previous findings in this report, there were significant strain variations seen for G6PD activity, hematocrit, hemoglobin, red blood cell count, mean corpuscular hemoglobin concentration, and osmotic fragility (Tables 25-27, 29, 34). Additionally, the WBC count (Table 28) and GSH (Tables 35) were higher for the C57L/J strain and the A/J strain ($p = \leq .001$ and $p \leq .000$ respectively).

A number of variables were effected by treatment and these included ENZ, WBC, HGB, MCV, and MCHC (Tables 25, 28-30, and 32). Additionally, analysis of variance revealed osmotic fragility to be affected by treatment at the $p = .081$ level (Table 34). However, when contrasts were performed on individual treatments and compared with controls, the level of significance increased and showed both A/J and C57L/J strains to have more osmotically fragile cells than controls when treated with 100 ppm chlorite (Figure 13). There were no strain versus treatment interactions according to analysis of variance. This is supported by data in Table 38 showing both the A/J and C57L/J strains to be similarly effected by treatment. Table 38 is a composite of the contrast results displaying which variables and strains were significantly effected by treatments 2, 3, or 4 when compared with controls. It is evident that all but two treatment effects were produced by the 100 ppm chlorite exposure. Hemoglobin appeared to increase significantly for the C57L/J strain at the 1.0 and 10.0 ppm chlorite exposure but not the 100 ppm level. The chlorite exposures at 1.0 and 10.0 ppm produce few or no treatment effects on the majority of parameters measured on this study.

Because the majority of treatment effects appeared in the 100 ppm chlorite treatment group, it seemed appropriate to compare the results of this group (Table 38) with similarly treated groups in Section 5 (Table 24) of this study. It was expected that the variables affected in both studies

would be the same since strains and treatments were equivalent. However, some treatments that appeared significant in one group did not appear significant in the other or were effected in opposite directions. This discrepancy may be due to the wide variability often seen in biological measurements. There were, however, three variables that were affected significantly by treatment with chlorite in both separate studies, (Tables 24, 38); and the effects were in the same direction. The variables showing replicated values were ENZ, MCV, and OSM. Based upon these replicated results, the conclusions are that treatment with 100 ppm chlorite for 30 days causes: (1) an increase in G6PD activity; (2) an increase in mean corpuscular volume; and (3) an increase in osmotic fragility. Both strains of mice react in a similar way to chlorite exposure when measured by these parameters and therefore are not strain versus treatment interactions.

SCANNING AND TRANSMISSION ELECTRON MICROGRAPHY OF ERYTHROCYTES FROM A/J AND C57L/J MICE EXPOSED TO 100 PPM CHLORITE FOR 30 DAYS

Heparinized whole blood from A/J and C57L/J mice treated with 100 ppm chlorite for 30 days, and from controls of each strain was preserved and prepared for electron microscopy according to procedures previously described. Scanning of the samples was performed by determining an area on the slide with a moderate density of erythrocytes and randomly selecting three areas to photograph. This function was performed by a technician blind to the identity and purpose of the samples. Each sample, therefore, yielded three photographs with approximately 700-1,000 cells at 6000X magnification. The treated mice appeared to have a larger number of acanthocytes*. To test this, a different technician was given the coded photographs and asked to determine the percentage of acanthocytes in each photograph. The results (Table 39) indicate that there is an increase in acanthocytes for both strains of mice treated with 100 ppm chlorite. Treatment causes the A/J strain to increase from 7.2 to 34.3 percent whereas the C57L/J strain increases from 4.4 to 19.1 percent acanthocytes. It is not clear what significance may be attributed to the presence of increased numbers of acanthocytes, but such spiculated cells usually indicate a disturbed erythrocyte lipid composition (26). Acanthocytes are seen in association with betalipoproteinemia (26,27), certain types of metastatic liver diseases (46), mild hemolytic anemia, and may be found in fresh, wet preparations (46). It is conjectured in this case that the chlorite may react with the cell membrane surface to disturb the lipid composition and thereby cause spicule formation.

Transmission electron micrography of A/J and C57L/J cells revealed the length of erythrocytes from cells of treated mice were longer than those of control mice (Table 40). The A/J strain showed an increase from 4.37 to 5.40 μ with treatment while the erythrocytes of C57L/J mice increased from 4.08 to 5.90 μ.

*An erythrocyte with 5 to 10 spiny projections or spicules that vary in length and thickness, and are irregularly spaced about the red blood cell.

TABLE 25

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR G6PD ACTIVITY (ENZ) OF A/J AND C57L/J MICE EXPOSED TO 0.0, 1.0, 10.0 OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - ENZ

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	417.91657	1	25696.69928	.000
S	33.98865	1	2089.88169	.000
T	.33802	3	6.92803	.000
ST	.00936	3	.19192	.902
ERROR	1.85403	114		

B. Descriptive Statistics

Variable Number	1	Maximum	2.88
Name	ENZ	Minimum	1.14
Number of Distinct Values .	102	Range	1.74
Number of Values Counted .	122	St. Dev.	.56
Number of Values not Counted	0		

Location Estimates

Mean	1.96
Median	2.27
Mode	NOT UNIQUE

*Number of A/J mice tested for treatment
**Number of C57L/J mice tested for treatment

C. Cell Means for Dependent Variable -ENZ

	Treatment (ppm)		Strain		
μ	ClO_2	N*	A/J	C57L/J	N**
1	0.0	16	6.10	1.88	14
2	1.0	12	6.05	1.74	11
3	10.0	12	5.90	1.82	11
4	100.0	23	6.50	2.13	23

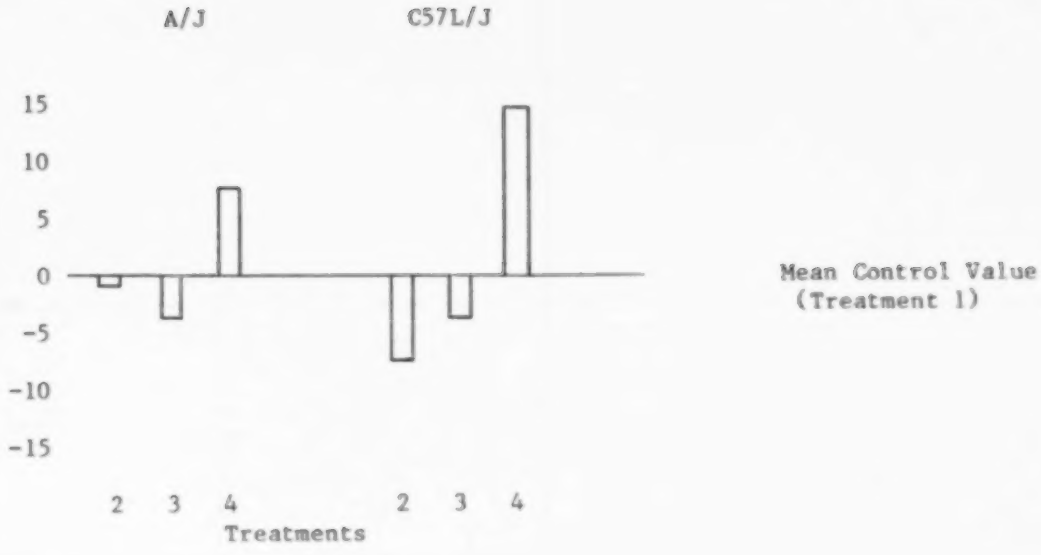

Figure 8. Percent change from controls of G6PD Activity for A/J and C57L/J mice exposed to 1.0, 10.0, and 100 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of this Difference Occurring by Chance Alone
A/J	1 vs. 4	.045
	2 vs. 4	.091
	3 vs. 4	.008
C57L/J	1 vs. 4	.022
	2 vs. 4	.002
	3 vs. 4	.036

TABLE 26

ANALYSIS OF VARIANCE, DESCRIPTIVE STATISTICS, AND CELL MEANS FOR RED BLOOD CELL COUNT (RBC) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - RBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	6530.75529	1	32740.92030	.000
S	28.82636	1	144.51646	.000
T	.71862	3	1.20089	.313
ST	1.07505	3	1.79654	.152
ERROR	21.34304	107		

B. Descriptive Statistics

Variable Number	2	Maximum	9.34	(Q3-Q1)/2	.470
Name . . . . . RBC		Minimum	6.28	St. Error	.064
Number of Distinct Values .	93	Range	3.06		.072
Number of Values Counted .	115	Variance	.483		
Number of Values not Counted	7	St. Dev.	.695		

Location Estimates

Mean	7.84
Median	7.87
Mode	NOT UNIQUE

C. Cell Means for 1st Dependent Variable- RBC

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	15	7-40	8.13	13
2	1.0	11	7-37	8.41	11
3	10.0	11	7-38	8.61	10
4	100.0	22	7-23	8.42	22

TABLE 27

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE-HCT OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS.

A. Analysis of Variance for 1 -ST Dependent Variable - HCT

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	59651.90179	1	25043.09497	.000
S	880.21257	1	138.07068	.000
T	25.94278	3	1.35647	.260
ST	19.82190	3	1.03642	.380
ERROR	682.13428	107		

B. Descriptive Statistics for the 1st Dependent Variable - HCT

Variable Number	3	Maximum	47.00	(Q3-Q1)	2.65
Name . . . . . HCT		Minimum	29.70	St. Error	.064
Number of Distinct Values .	76	Range	17.30		.072
Number of Values Counted .	115	Variance	14.84		
Number of Values not Counted	7	St. Dev.	3.85		

Location Estimates

Mean	38.93
Median	38.20
Mode	NOT UNIQUE

C. Cell Means for 1st Dependent Variable - HCT

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	15	36.16	40.55	13
2	1.0	10	38.77	41.58	11
3	10.0	11	35.77	42.49	10
4	100.0	22	36.41	42.61	22

TABLE 28

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR WHITE BLOOD CELL COUNT (WBC) OF A/J AND C57L/J CONTROLS AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, or 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - WBC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	834.34213	1	1874.03069	.000
S	5.46578	1	12.27678	.001
T	7.38528	3	5.52941	.001
ST	.31971	3	.23937	.869
ERROR	7.63775	107		

B. Descriptive Statistics for Dependent Variable - WBC (Square Root)

Variable Number	4	Maximum	4.9699095	(Q3-Q1)	.4914141
Name . . . . . WBC		Minimum	1.4594520	St. Error	.0678801
Number of Distinct Values .	90	Range	3.5104575		.1027886
Number of Values Counted .	115	Variance	.5298860		
Number of Values not Counted	7	St. Dev.	.7279327		

Location Estimates

Mean	2.7311456
Median	2.7018512
Mode	1.9235384

C. Cell Means for Dependent Variable - WBC

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	15	6.4	9.12	13
2	1.0	11	7.73	10.24	11
3	10.0	11	7.50	11.15	10
4	100.0	22	5.19	6.71	22

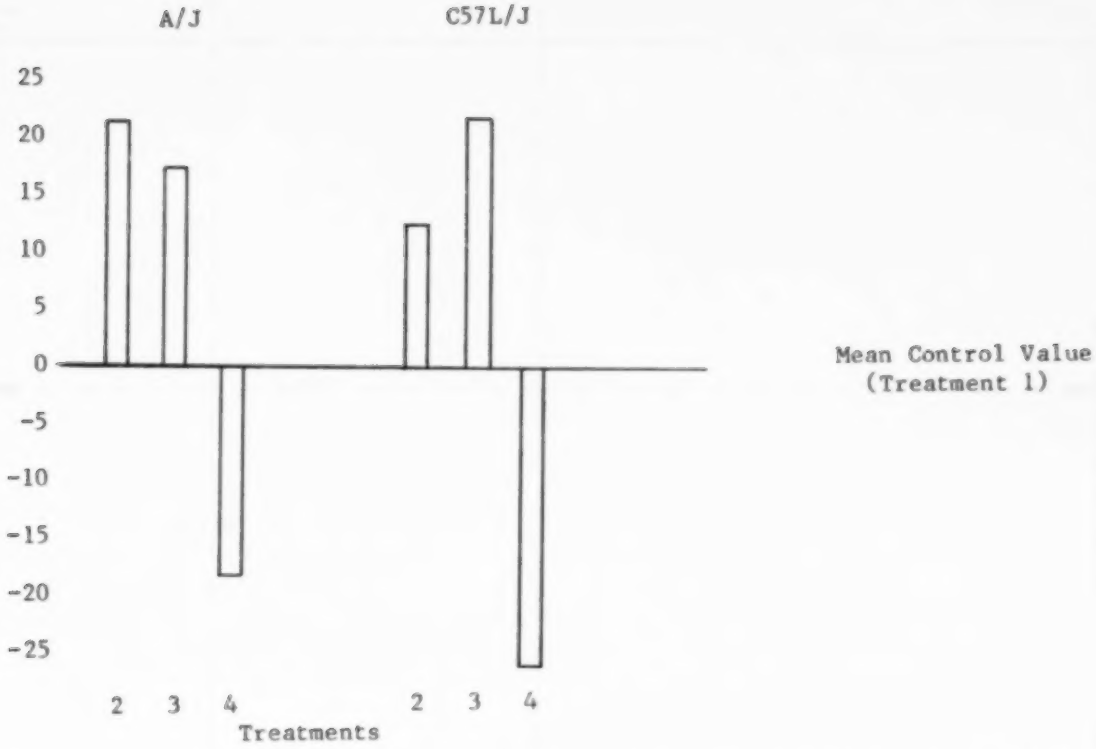

Figure 9. Percent change from controls of White Blood Cell Counts (WBC) for A/J and C57L/J Mice Exposed to 1.0, 10,0, and 100.0 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of this Difference Occurring by Chance Alone
A/J	2 vs. 4	.066
	3 vs. 4	.070
	1 vs. 4	.071
C57L/J	2 vs. 4	.003
	3 vs. 4	.022

TABLE 29

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - FOR HEMOGLOBIN (HGB) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - HGB

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	22694.82517	1	42239.07337	.000
S	68.66551	1	127.79863	.000
T	12.14370	3	7.53386	.000
ST	2.46240	3	1.52766	.211
ERROR	61.25158			

B. Descriptive Statistics for the 1st Dependent Variable - HGB

Variable Number	5	Maximum	17.0000000	(Q3-Q1)/2	.7500000
Name . . . . . HGB		Minimum	11.7000000	St. Error	.0994583
Number of Distinct Values .	46	Range	5.3000000		.1299039
Number of Values Counted .	122	Variance	1.2068184		
Number of Values not Counted	0	St. Dev.	1.0985528		

Location Estimates

Mean	14.0954918
Median	14.0500000
Mode	NOT UNIQUE

C. Cell Means for Dependent Variable - HGB

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	16	13.53	14.61	14
2	1.0	12	13.55	15.36	11
3	10.0	12	13.62	15.44	11
4	100.0	23	13.00	14.54	23

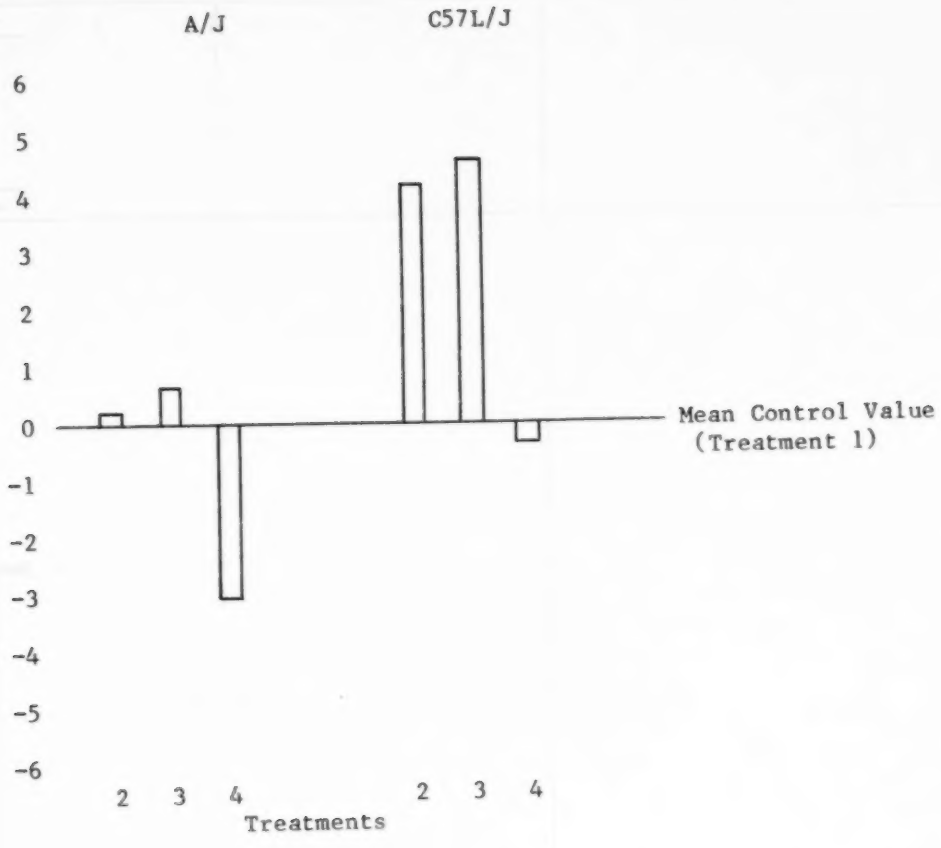

Figure 10. Percent change from controls of Hemoglobin (HGBO for A/J and C57L/J mice exposed to 1.0, 10.0, and 100.0 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasts	Probability of this Difference Occurring by Chance Alone
A/J	1 vs. 4	.010
	2 vs. 4	.042
	3 vs. 4	.007
C57L/J	1 vs. 2	.025
	1 vs. 3	.017
	2 vs. 4	.012
	3 vs. 4	.008

TABLE 30

ANALYSIS OF VARIANCE for 1-ST DEPENDENT VARIABLE FOR MEAN CORPUSCULAR VOLUME (MCV) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - MCV

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	275065.75733	1	64166.62189	.000
S	11.81293	1	2.75569	.100
T	52.95621	3	4.11783	.008
ST	3.52653	3	.27422	.844
ERROR	488.68868	114		

B. Descriptive Statistics for 1st Dependent Variable - MCH

Variable Number	6	Maximum	55.2600000	(Q3-Q1)/3	1.6300000
Name . . . . . MCV		Minimum	45.2600000	St. Error	.1942036
Number of Distinct Values .	115	Range	10.0000000		.2655812
Number of Values Counted .	122	Variance	4.6012347		
Number of Values not Counted	0				

Location Estimates

Mean	49.6400000
Median	49.7400000
Mode	NOT UNIQUE

C. Cell Means for 1st Dependent Variable -MCV

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	16	48.91	49.89	14
2	1.0	12	48.70	49.47	11
3	10.0	12	48.58	49.25	11
4	100.0	23	50.39	50.544	23

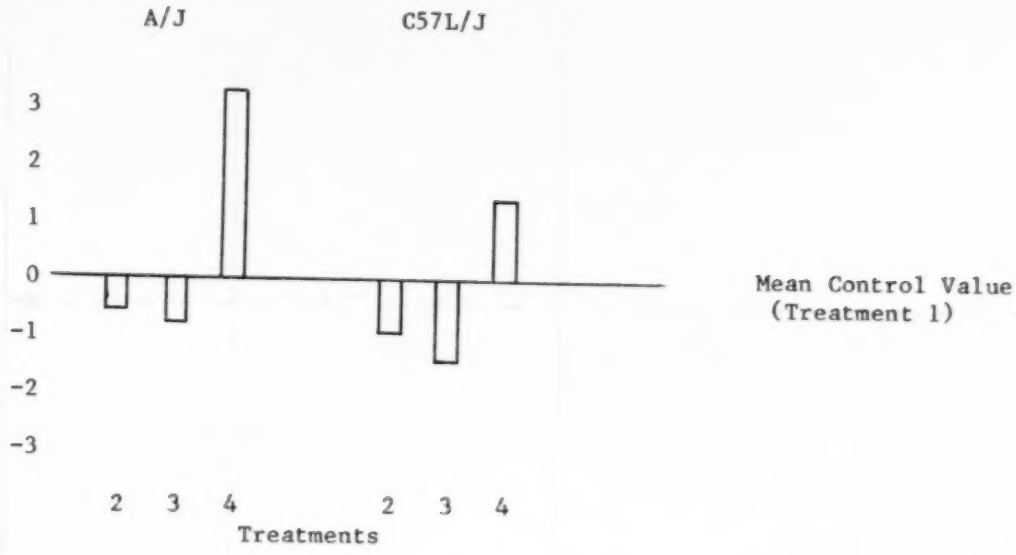

Figure 11. Percent change from controls of Mean Corpuscular Volume (MCV) for A/J and C57L/J mice exposed to 1.0, 10.0, and 100.0 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of this Difference Occurring by Chance Alone
	1 vs. 4	.024
A/J	3 vs. 4	.030
C57L/J	3 vs. 4	.031

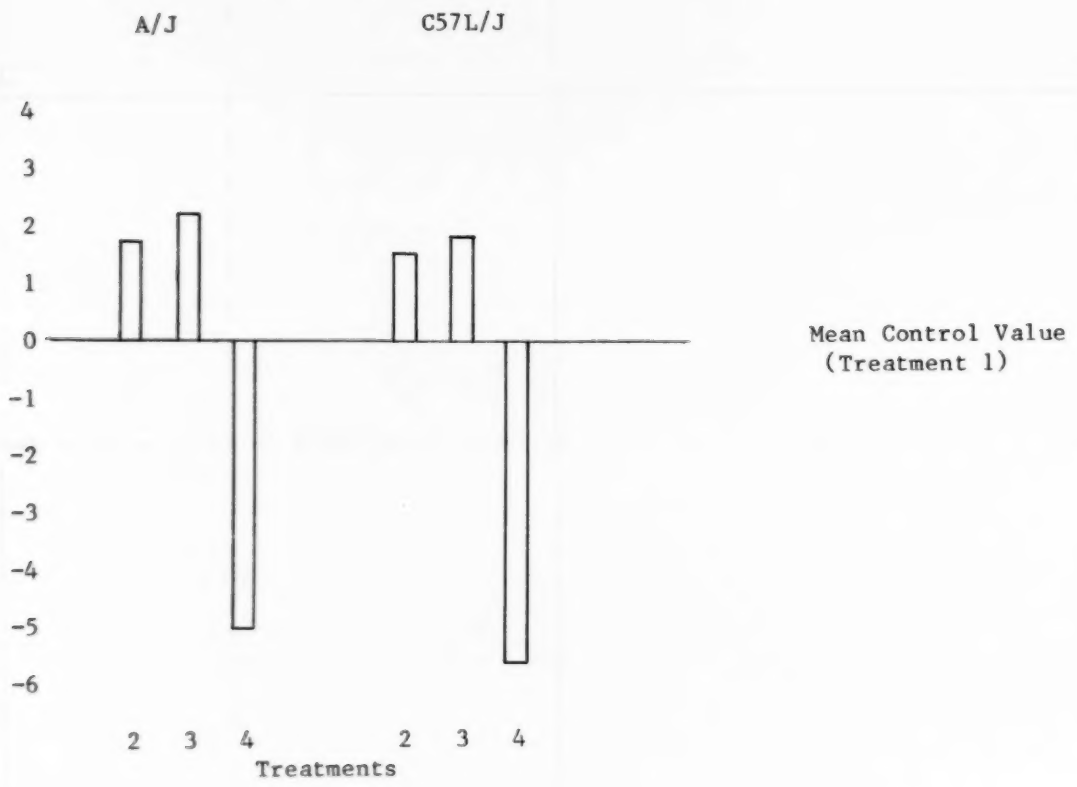

Figure 12. Percent change from controls of Mean Corpuscular Hemoglobin Concentration (MCHC) for A/J and C57L/J mice exposed to 1.0, 10.0, and 100.0 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasts	Probability of this Difference Occurring by Chance Alone
A/J	1 vs. 4	.085
	2 vs. 4	.053
	3 vs. 4	.030
C57L/J	1 vs. 4	.081
	2 vs. 4	.036
	3 vs. 4	.039

TABLE 31

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - FOR MEAN CORPUSCULAR HEMOGLOBIN (MCH) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - MCH

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	37595.60662	1	20908.87489	.000
S	6.49976	1	3.61486	.060
T	12.89371	3	2.39029	.072
ST	.79345	3	.14709	.931
ERROR	204.9790			

B. Descriptive Statistics for 1st Dependent Variable - MCH

Variable Number	7	Maximum	22.0500000	(Q3-Q1)/2	.7350000
Name . . . . . MCH		Minimum	15.8400000	St. Error	.1240337
Number of Distinct Values .	105	Range	6.2100000		.1356774
Number of Values Counted .	122	Variance	1.8768930		
Number of Values not Counted	0				

Location Estimates

Mean	18.2031967
Median	17.9300000
Mode	17.6800000

C. Cell Means for 1st Dependent Variable - MCH

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	16	18.49	18.18	14
2	1.0	12	18.71	18.29	11
3	10.0	12	18.76	18.27	11
4	100.0	23	18.14	17.43	23

TABLE 32

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - FOR MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, and 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - MCHC

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	154325.11177	1	14834.79318	.000
S	60.11750	1	5.77891	.018
T	153.83165	3	4.92912	.003
ST	.12656	3	.00406	1.000
ERROR	1185.93246	114		

B. Descriptive Statistics for 1st Dependent Variable -MCHC

Variable Number	8	Maximum	44.6700000	(Q3-Q1)/2	2.3900000
Name . . . . . MCHC		Minimum	30.6200000	St. Error	.3090592
Number of Distinct Values .	112	Range	14.0500000		.4965215
Number of Values Counted .	122	Variance	11.6531431		
Number of Values Not Counted	0	St. Dev.	3.4136700		

Location Estimates

Mean	36.7591803
Median	36.5250000
Mode	NOT UNIQUE

C. Cell Means for 1st Dependent Variable - MCHC

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	16	37.86	36.50	14
2	1.0	12	38.52	37.06	11
3	10.0	12	38.67	37.15	11
4	100.0	23	36.09	34.57	23

TABLE 33

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR RETICULOCYTES (RET) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, or 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - RET

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	273.86982	1	350.06424	.000
S	1.35756	1	1.73013	.191
T	7.73180	3	3.29430	.023
ST	1.55814	3	.66388	.576
ERROR	82.14587	105		

B. Descriptive Statistics

Variable Number	9	Maximum	4.5000000	(Q3-Q1)/2	.6000000
Name . . . . . RET		Minimum	0.0000000	St. Error	.0860251
Number of Distinct Values .	32	Range	4.5000000		.1154701
Number of Values Counted .	113	Variance	.8362358		
Number of Values not Counted	9	St. Dev.	.9144593		

Location Estimates

Mean	1.5645018
Median	1.6000000
Mode	2.0000000

C. Cell Means

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	15	1.45	1.43	13
2	1.0	11	1.69	1.64	10
3	10.0	11	2.21	1.90	10
4	100.0	22	1.61	1.07	24

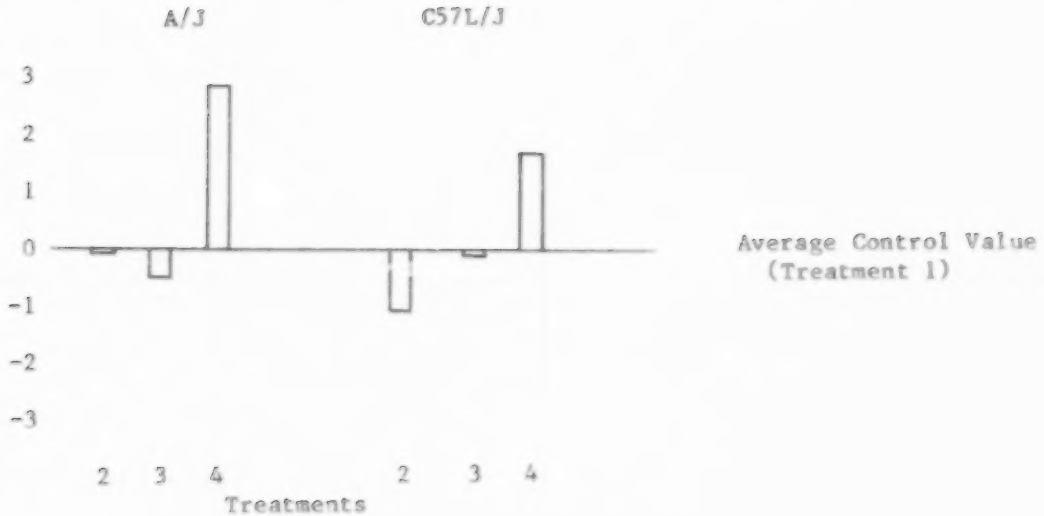

Figure 13. Percent change from control of Osmotic (OSM) for A/J and C57L/J mice exposed to 1.0, 10.0, and 100.0 ppm chlorite for 30 days.

CONTRASTS

Strain	Treatments Contrasted	Probability of this Difference Occurring of Chance Alone
A/J	1 vs. 4	.056
C57L/J	1 vs. 4	.018

TABLE 34

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR OSMOTIC FRAGILITY (OSM) OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - OSM

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	18.64819	1	60513.73139	.000
S	.00603	1	19.57041	.000
T	.00215	3	2.32335	.081
ST	.00021	3	.22973	.875
ERROR	.02589	84		

B. Descriptive Statistics

Variable Number	12	Maximum	.487	(Q3-Q1)/2	.013
Name . . . . . OSM		Minimum	.404	St. Error	.0020242
Number of Distinct Values .	49	Range	.083		.0028868
Number of Values Counted .	92	Variance	.0003770		
Number of Values not Counted	0	St. Dev.	.019		

Location Estimates

Mean	
Median	117.1000000
Mode	119.3000000

C. Cell Means

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	11	112.87	119.96	10
2	1.0	11	108.46	120.82	11
3	10.0	11	110.06	125.87	10
4	100.0	11	103.83	118.59	10

TABLE 35

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR GSH OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - GSH

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	1122892.44303	1	7900.02707	.000
S	3315.90414	1	23.32880	.000
T	530.15015	3	1.24328	.300
ST	237.64753	3	.55732	.645
ERROR	10944.61036	77		

B. Descriptive Statistics for the Dependent Variable - GSH

Variable Number	12	Maximum	139.1000000	(Q3-Q1)/2	7.4500000
Name . . . . . GSH		Minimum	66.2000000	St. Error	1.4510282
Number of Distinct Values .	75	Range	72.9000000		1.7897867
Number of Values Counted .	85	Variance	178.9660448		
Number of Values not Counted	7	St. Dev.	13.3778191		

Location Estimates

Mean	114.8329412
Median	117.1000000
Mode	119.3000000

C. Cell Means

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	11	112.87	119.96	10
2	1.0	11	108.46	120.82	11
3	10.0	11	110.06	125.87	10
4	100.0	11	103.83	118.59	10

TABLE 36

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR WT OF A/J AND C57L/J CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - WT (log)

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	86.87742	1	381.42599	.000
S	.01938	1	.08507	.772
T	1.28012	3	1.87341	.146
ST	.85860	3	1.25654	.300
ERROR	11.16073	49		

B. Descriptive Statistics for the Dependent Variable - WT (Log)

Variable Number	13	Maximum	.4970000	(Q3-Q1)/2	.0517500
Name . . . . . WT		Minimum	-.2890000	St. Error	.0125917
Number of Distinct Values .	77	Range	.7860000		.0066395
Number of Values Counted .	88	Variance	.0139525		
Number of Values not Counted	4	St. Dev.	.1181208		

Location Estimates

Mean	.0262159
Median	.0220000
Mode	NOT UNIQUE

C. Cell Means for Dependent Variable - WT (Log)

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	10	-1.62	-1.35	7
2	1.0	5	-1.00	-1.16	7
3	10.0	7	-1.00	-1.36	7
4	100.0	9	-1.35	-1.25	5

TABLE 37

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE FOR WATER CONSUMED IN MILLILITERS PER DAY (WAT) OF A/J AND C57LOJ CONTROLS, AND TEST MICE EXPOSED TO 0.0, 1.0, 10.0, OR 100.0 PPM OF SODIUM CHLORITE FOR 30 DAYS

A. Analysis of Variance for 1 -ST Dependent Variable - WAT

SOURCE	SJM OF SQUARES	DEGREES OF FREEDOM	F	PROB. F EXCEEDED
MEAN	26154.19592	1	6837.98527	.000
S	17.71331	1	4.63113	.034
T	14.53322	3	1.26656	.291
ST	8.69583	3	.75784	.521
ERROR	321.28652	84		

B. Descriptive Statistics for the Dependent Variable -WAT

Variable Number	14	Maximum	21.4000000	(Q3-Q1)/2	1.4500000
Name . . . . . WAT		Minimum	12.9000000	St. Error	.2077410
Number of Distinct Values .	57	Range	8.5000000		.2309402
Number of Values Counted .	92	Variance	3.9703810		
Number of Values not Counted	0	St. Dev.	1.9925815		

Location Estimates

Mean	16.8576087
Median	16.8500000
Mode	17.1000000

C. Cell Means for Dependent Variable - WAT

	Treatment (ppm)		Strain		
#	ClO_2	N	A/J	C57L/J	N
1	0.0	12	16.32	16.49	11
2	1.0	12	16.36	17.18	11
3	10.0	12	16.57	18.43	11
4	100.0	12	16.50	17.15	11

TABLE 38

VARIABLES AND STRAINS FOR WHICH TREATMENTS 2-4 (1, 10, AND 100 PPM CHLORITE) PRODUCED SIGNIFICANT EFFECTS COMPARED WITH CONTROLS

	A/J Strain			C57L/J Strain		
	1.0*	10.0	100	1.0	10.0	100
+ENZ	-	-	.04>**	-	-	.022>
WBC	-	-	-	-	-	.071<
HGB	-	-	.010<	.025>	.017>	-
+MCV	-	-	.024>	-	-	-
MCHC	-	-	.085<	-	-	.081<
+OSM	-	-	.056>	-	-	.018>

* ppm of sodium chlorite used on treatment groups.

** direction of change from control: > = higher mean value than control; < = lower mean value than control.

+ variables which were effected significantly and in a similar direction by 100 ppm chlorite exposure as in a previous experiment (see Table 24).

TABLE 39

PERCENTAGE OF ACANTHOCYTES IN SCANNING ELECTRON MICROGRAPHS OF A/J AND C57L/J ERYTHROCYTES TREATED WITH 100 PPM OF CHLORITE FOR 30 DAYS

	TREATMENT	
STRAIN	0.00 ppm Chlorite	100 ppm Chlorite
A/J	7.2 ≠ 6.9%	34.3 ≠ 10.9%
C57L/J	3.7 ≠ 3.6%	15.9 ≠ 11.8%

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